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### DESCRIPTIONS OF NORTH AMERICAN SENECIONE &1

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The following descriptions and notes are the results obtained from a critical study of material in several herbaria during the preparation of a monograph of the North American species of the genus Senecio. Some of the species here described have been in manuscript a number of years and a few of them have been withheld from publication, because of incomplete specimens, hoping that additional material might be brought together In many cases supplementary and subbefore publication. stantiating material has been obtained from which it is now possible to make fairly complete diagnoses. In one or two instances a reconsideration of certain natural groups within the genus, in the light of recent collections, has made it possible to combine forms which formerly were taken to represent distinct species. Very few new species have resulted from recent collections, but there are still many regions, particularly in Central America, which are inadequately explored. The writer would welcome material in this genus from any part of North America in order that the geographical range of species may be recorded as accurately as possible in his forthcoming monograph. The sections indicated in parentheses immediately following the generic name are in accordance with my preliminary paper to which reference is made under the species.

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Senecio (§ Aurei) hyperborealis Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

S. resedifolius Hook. Fl. Bor. Am. 1: 333. pl. 117. 1833, not Less. Herbaceus perennis; caule simplice vel ramoso suberecto 1-2 dm. alto plus minusve foliaceo juventate glabro vel parce floculoso-tomentuloso sæpe ad basin et in axillis foliorum persistenter lanato-tomentoso; foliis inferioribus petiolatis indivisis vel plerumque irregulariter lyrato-pinnatifidis 4-10 cm. longis 1-2.5 cm. latis, lobis remotis; foliis superioribus multum reductis sessilibus et bracteiformibus; capitulis paucis terminalibus radiatis 10-12 mm. altis 2-3.5 cm. (radii inclusis) diametro; floribus femineis 10-12, ligulis flavis 10-12 mm. longis ca. 2 mm. latis; disci flosculis numerosis; achæniis sæpe paulo hispidulis.

Specimen examined:

Canada: Arctic America, Hooker (Gray Herb.), TYPE.

Var. columbiensis (Gray) Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

S. resedifolius var. columbiensis Gray, Syn. Fl. 12: 390. 1884. Habitu formae typicæ; capitulis heterogamis, ligulis floris femineis quam squamis involucri paulo brevioribus; achæniis glabris.

Specimen examined:

British Columbia: Mucklung River, 25 July, 1882, Mr. Mackay (Gray Herb.).

Senecio (§ Lobati) prolixus, comb. nov.

S. diffusus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen, not Linn. f.

Herbaceus perennis glabrus vel in axillis foliorum albotomentosus; caule tereti striato simplici vel ramoso erecto 2-5 dm. alto; foliis petiolatis vel sessilibus inferioribus lyrato-pinnatifidis petiolo incluso usque ad 15 cm. longis 1.5-5 cm. latis utrinque glabris, segmentis lateralibus oblongo-cuneatis cum sinis altis rotundatis disjunctis granditer dentatis, superioribus remotis sessilibus pinnatifidis sursum multum reductis; inflorescentiis laxe corymboso-cymosis 1-2.5 dm. diametro; capitulis circiter 1 cm. altis radiatis; involucris campanulatis parce calyculatis glabris; involucri squamis plerumque 21 lanceolatis vel lineari-lanceolatis 5-6 mm. longis acuminatis acutis; flosculis liguliferis ca. 13, ligulis oblongis 5-6 mm. longis flavis;

floribus disci numerosis 50-60; achæniis maturitate 2-3 mm. longis striatis glabris.

Specimens examined:

California (?): "Mohave Region," April-May, coll. of 1884, J. G. Lemmon, 3130 (Gray Herb.), Type.

Arizona: Wickenburg, valley of the Hassayampa River, April, 1876, Dr. Edward Palmer, 614 (Gray Herb. and Mo. Bot. Gard. Herb.).

The specimens cited may be looked for in herbaria under S. multilobatus Torr. & Gray, to which the species here proposed is related, but from which it differs in well developed specimens in the outline and size of the leaves, loose inflorescence, and larger heads with 21 instead of 13 involucral bracts. S. prolixus has rather more the aspect of S. Breweri Davy.

Senecio (§ Tomentosi) appendiculatus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen. S. neo-mexicanus Gray, Proc. Am. Acad. 19: 55. 1883, in part; Syn. Fl. 12: 392. 1884, in part, as to plant of Thurber.

Herbaceus perennis ubique plus minusve albo-tomentosus; caulibus subcæspitosis erectis 1.5–3 dm. altis striatis sæpe foliaceis; foliis radicalibus oblanceolatis vel oblongo-obovatis petiolo incluso 3.5–10 cm. longis 0.5–2 cm. latis dentatis ad basin in petiolum paulatim angustatis integris, eis caulinis petiolatis vel sessilibus 2–7 cm. longis ad basin plerumque ampliatis irregulariter dentatis subamplexicaulibusque; inflorescentiis terminalibus corymboso-cymosis 6–12-cephalis; capitulis 10–12 mm. altis radiatis; involucris campanulatis minute calyculatis; involucri squamis plerumque 21 lanceolatis 5–7 mm. longis acutis sparsissime tomentulosis; flosculis liguliferis ca. 13, ligulis flavis; floribus disci numerosis ca. 70; achæniis glabris.

Specimens examined:

New Mexico: Mule Spring, May, 1851, Geo. Thurber, 280 (Gray Herb.), TYPE; Organ Mountains, Dona Ana Co., 25 April, 1907, E. O. Wooton, 3370 (Mo. Bot. Gard. Herb.).

This species is related to S. neo-mexicanus Gray, to which it has been usually referred, but from which it differs in having a more leafy stem, undivided leaves, and with the stem-leaves commonly ampliated into a more or less dentate half-clasping base, and finally in having glabrous instead of hirtellous achenes.

Senecio (§ Tomentosi) convallium Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

Herbaceus perennis ubique sericeo-pubescentes; caulibus cæspitosis erectis 3 dm. altis; foliis inferioribus rosulatis petiolatis elliptico-lanceolatis vel oblongo-oblanceolatis 2.5–6 cm. longis 5–12 mm. latis acutis integris vel supra mediam partem paucidentatis basi longe cuneatis integriusculis juventate utrinque sericeo-pubescentibus ætate supra plus minusve glabratis, foliis superioribus spathulato-oblanceolatis angusti-petiolatis; inflorescentiis corymboso-cymosis paucicapitatis; capitulis circiter 1 cm. altis subradiatis; involucri bracteis 13–15 lineari-attenuatis 7–9 mm. longis acutis sparse sericeo-tomentulosis; floribus femineis subligulatis; floribus disci 30–35; achaeniis 3.5 mm. longis striatis glabris.

Specimen examined:

Utah: Rabbit Valley, altitude 2130 m., August, 1875, L. F. Ward, 704 of the "U. S. Geological and Geographical Survey of the Territories" (Gray Herb.), TYPE.

The species here characterized has been hitherto confused with S. canus Hook., from which it is readily distinguished by the subsericeous pubescence and technical characters of the head.

Senecio (§ Tomentosi) kernensis Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

Herbaceus perennis ubique dense lanato-tomentosus; caule tereti erecto ca. 1 dm. alto; foliis inferioribus rosulatis petiolatis elliptico-oblongis vel oblongo-rotundatis 1–3 cm. longis 3–10 mm. latis apice obtusis vel rotundatis basi abrupte angustatis vel subtruncatis utrinque dense lanato-tomentosis, marginibus integris vel subcrenato-dentatis revolutisque, foliis superioribus bracteiformibus multum reductis; inflorescentiis terminalibus corymboso-cymosis paucicapitatis; capitulis 8–10 mm. altis radiatis 5–8 mm. (radii exclusis) diametro parce calyculatis; involucri squamis ca. 13 lineari-lanceolatis 5–6 mm. longis acutis floccosotomentulosis subglabratis; achaeniis glabris.

Specimen examined:

California: South Fork of Kern River, altitude 3760 m., September, 1875, Dr. J. T. Rothrock, 334 of the "Explorations and Surveys west of the 100th Meridian" (Gray Herb.), TYPE.

Senecio (§ Tomentosi) macropus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

S. arizonicus Gray, Syn. Fl. 12: 392. 1884, in part, as to plant of Rusby.

Radix robusta in sicco 2.5 cm. diametro; caulibus erectis usque ad 7.5 dm. altis glabris vel in axillis foliorum albo-tomentulosis striatis plus minusve purpurascentibus; foliis radicalibus petiolatis lyrato-pinnatifidis petiolo incluso 10–14 cm. longis 4–5 cm. latis, segmentis paucijugis inæqualibus terminali majore ovato-oblongis 5–6 cm. longis grosse dentatis, ceteris cuneatis et dentatis vel linearibus et integris; foliis caulinis remotis sessilibus pinnato-lobatis semiamplexicaulibusque sursum sensim reductis; inflorescentiis terminalibus corymboso-cymosis; capitulis ca. 1 cm. altis radiatis, ligulis flavis; involucris campanulatis minute calyculatis; involucri squamis circiter 21 lineari-lanceolatis 6.5–8 mm. longis acutis glabris maturitate retrorsis; floribus disci numerosis; achæniis glabris.

Specimen examined:

Arizona: without definite locality, coll. of 1883, H. H. Rusby, 175 (Gray Herb.), TYPE.

Professor Rusby's plant was referred by Dr. Gray to S. arizonicus Greene, but from the very large root, the sublyrate, smooth and even somewhat glaucous radical leaves, and nearly naked stem it seems amply distinct.

Senecio (§ Tomentosi) oreophilus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen.

S. neo-mexicanus Gray, Proc. Am. Acad. 19: 55. 1883, in part; Syn. Fl. 12: 392. 1884, in part, as to plant of Greene.

Herbaceous perennis juventate ubique tomentulosus denique plus minusve glabratus; caule tereti erecto striato 2–3 dm. alto subnudo 2–3-bracteato; foliis rosulatis petiolatis oblongo-oblanceolatis vel oblongo-cuneatis petiolo incluso 3–10 cm. longis 0.7–2.5 cm. latis supra mediam partem crenato-dentatis basi in petiolum sensim angustatis integriusculis juventate utrinque albo-tomentulosis mox glabratis; bracteis caulinis linearibus apice basique parum ampliatis dentatisque; inflorescentiis laxe corymboso-cymosis usque ad 1 dm. diametro; capitulis 10–12 mm. altis calyculatis radiatis; involucris campanulatis basi tomentulosis ceteris glabris; involucri squamis plerumque 21

lanceolatis 6.5–8 mm. longis acutis; flosculis liguliferis ca. 12, ligulis oblongis 8 mm. longis 3 mm. latis 4–5-nerviis; floribus disci numerosis ca. 50; achæniis in angulis sursum hispidulis.

Specimen examined:

New Mexico: Pinos Altos Mountains, 6 May, 1880, Edward Lee Greene (Gray Herb.), TYPE.

A plant similar in habit to S. neo-mexicanus Gray, to which Dr. Greene's specimen was referred by Professor Gray in establishing that species. A careful study of all the original material, which has been made possible through the courtesy of Dr. B. L. Robinson, has shown that the S. neo-mexicanus of Dr. Gray consisted of at least three recognizably distinct forms of which Wright's No. 1415, as the first specimen cited, must be taken as the type. With the Wright plant several specimens at hand are almost the exact counterpart. The Greene plant in question, namely S. oreophilus, differs in several important particulars, notably in its essentially naked stem, oblong-cuneate leaves with subentire or sinuate-dentate margin, and a marked tendency for the foliage to become glabrous with age.

Senecio (§ Tomentosi) oreopolus Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen. Plate 11.

Herbaceus perennis ubique albo-tomentosus; caulibus cæspitosis simplice vel ramosis 0.8–3 dm. altis; foliis inferioribus petiolatis ovato-ellipticis vel elliptico-lanceolatis vel rarius subobovatis 0.8–3.5 cm. longis 5–18 mm. latis obtusis vel supra mediam partem paucidentatis basi abrupte vel longe cuneatis integriusculis juventate utrinque albo-tomentosis ætate supra paululo subinde glabratis, petiolatis 1–6.5 cm. longis, foliis supremis grosse reductis petiolatis vel sessilibus integris vel rarius irregulariter dentatis basi sæpe expansis et subauricularibus; inflorescentiis corymboso-cymosis; capitulis plerumque ca. 1 cm. (8–14 mm.) altis radiatis parce calyculatis; involucri squamis plerumque 13 (9–13) lanceolatis vel lineari-lanceolatis 5–7 mm. longis acutis glabris vel leviter tomentulosis; flosculis liguliferis 5–13; floribus disci 20–30; pappi setis albis bracteis involucri longioribus; achæniis 3–3.5 mm. longis glabris.

Specimens examined:

California: Rock Creek Cañon, Basin of the Upper Kern River. Tulare Co., altitude 3050 m., July, 1904, H. M. Hall & H. D. Babcock, 5526 (Gray Herb.), TYPE; Natural Bridge, Volcano Creek, Basin of the Upper Kern River, altitude 2285 m., July, 1904, H. M. Hall & H. D. Babcock, 5433 (Grav Herb.); gravelly slopes, Little Kern River, altitude 3045-3350 m., April-September, 1897, C. A. Purpus, 5240 (Gray Herb. and Mo. Bot. Gard. Herb.); Castle Peak, near the highest point, altitude 2740 m., 5 August, 1903, A. A. Heller, 7102 (Gray Herb. and Mo. Bot. Gard. Herb.); Sierra Nevada, coll. of 1875, John Muir, 4452 (Mo. Bot. Gard. Herb.); near the summit of Silver Mountain, altitude 3350 m., coll. of 1863, W. H. Brewer, 2050 (Gray Herb.); Ebbett's Pass, W. H. Brewer, 2005 (Gray Herb.); Sonora Pass, W. H. Brewer, 2686 (Gray Herb.); Mono Pass, coll. of 1866, H. N. Bolander, 6140 (Gray Herb.).

Nevada: Mt. Rose, Washoe Co., altitude 3200 m., 26 August, 1911, A. A. Heller, 9882 (Mo. Bot. Gard. Herb.).

Forma aphanactis, forma nova.

Caulis circiter 1 dm. altus; foliis petiolo incluso 1.5–2.5 cm. longis 5–7 mm. latis; capitulis discoideis.

Specimen examined:

California: mountain peak near Sonora Pass, altitude 3200 m., coll. of 1863, W. H. Brewer, 1905 (Gray Herb.), TYPE.

Senecio (§ Tomentosi) Wrightii Greenm. Monogr. Senecio, pt. 1, 24. 1901; in Engl. Bot. Jahrb. 32: 20. 1902, nomen. S. fastigiatus Gray, Pl. Wright. ii. 99. 1853, not Nutt.

Herbaceus perennis ubique subtomentosus; caule erecto 1-4 dm. alto foliato; foliis oblongo-oblanceolatis vel lanceolatis indivisis et integris vel supra mediam partem paucidentatis juventate albo-tomentosis plus minusve glabratis, inferioribus basi integriusculis in petiolum sensim angustatis, eis caulinis sessilibus basi sæpius ampliatim et irregulariter dentatis amplexicaulibusque; inflorescentiis terminalibus subcorymbosocymosis multicapitatis; capitulis 8-10 mm. altis minute calyculatis radiatis; involucris campanulatis basi subincrassatis tomentosis, bracteis involucri plerumque 13 lanceolatis 5-7 mm. longis acutis tomentulosis; flosculis liguliferis 6-8, ligulis anguste

oblongis ca. 8 mm. longis 4-5-nerviis; floribus disci ca. 30; achaeniis glabris.

Specimens examined:

New Mexico: ravines between the copper mines and the Mimbres, October, 1851, Charles Wright, 1289 (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE; Santa Rita del Cobre, 22 September, 1880, E. L. Greene (Mo. Bot. Gard. Herb.); among spruce, Lookout Mine, Sierra Co., altitude 2680 m., O. B. Metcalfe, 1179 (Mo. Bot. Gard. Herb.).

Senecio (§ Amplectentes) subauriculatus Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen. Plate 14.

Herbaceus perennis; caule erecto ramoso striato glabro; foliis in partibus superioribus caulinis anguste lanceolatis 5–15 cm. longis 0.5–1.5 cm. latis acuminatis acutis integris vel remote apiculato-denticulatis sessilibus et auriculo-semiamplexicaulibus vel basi in petiolum sensim angustatis et subdecurrentibus membranceis supra glabris juventate subtus floccosotomentosis denique plus minusve glabratis; inflorescentiis terminalibus laxe subcorymboso-cymosis; pedunculis bracteatis, bracteis lineari-attenuatis; capitulis radiatis 12–14 mm. altis heterogamis; involucris campanulatis calyculatis albo-floccosotomentulosis, bracteolis calyculatis linearis acutis suberosomarginatis; involucri squamis plerumque 21 lineari-lanceolatis ca. 1 cm. longis acutis et atro-penicillatis; flosculis liguliferis ca. 13, ligulis oblongis flavibus; floribus disci numerosis (50–60); pappi setis albis; achæniis pubescentibus.

Specimen examined:

Mexico: State of Oaxaca, mountains southeast of Miahuatlan, altitude 2750-3170 m., coll. of 1895, E. W. Nelson, 2526 (Gray Herb.), Type.

A well marked species related to S. Warszewiczii A. Br. & Bouché and to S. prionopterus Rob. & Greenm.

Senecio (§ Mulgedifolii) alatipes Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen.

Herbaceus perennis ubique glabrus; caule tereti striato erecto 1 m. vel ultra alto; foliis parte inferiori ignotis, eis caulinis petiolatis vel sessilibus amplexicaulibusque oblongo-ovatis vel oblongo-lanceolatis 0.5–1.5 dm. longis 2–5 cm. latis acutis vel

acuminatis indivisis vel subpanduriformibus utrinque glabris subtus pallidoribus, margine irregulariter calloso-dentatis; petiolis usque ad 12 cm. longis anguste alatis; inflorescentiis
terminalibus paniculatis; capitulis 8–10 mm. altis discoideis
20–25-floris; involucris anguste campanulatis calyculatis glabris;
involucri squamis plerumque 13 lineari-lanceolatis acutis penicillatis ca. 6 mm. longis; achæniis striatis glabris.

Specimen examined:

Mexico: State of Chiapas, between Teneapa and Yajalon, altitude 900-1520 m., 13 October, 1895, E. W. Nelson, 3277 (U. S. Nat. Herb., fragments and tracing in Gray Herb.), TYPE. Senecio (§ Mulgedifolii) callosus Schz. Bip. in Flora 28: 498, 1845.

S. eximius Hemsl. Biol. Cent.-Am. Bot. 2: 239. 1881, as to synonomy.—S. doratophyllus Hemsl. l. c., in part, as to Bourgeau's No. 1086, not Benth.—S. viejensis and S. latipes Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen.—Cacalia Toluccana DC. Prodr. 6: 328. 1837.—C. prenanthoides Gray, Proc. Am. Acad. 19: 53. 1883, in part, as to Bourgeau's No. 1086, not HBK.—Erechthites runcinata Hemsl. Biol. Cent.-Am. Bot. 2: 234. 1881, in part, as to Bourgeau's No. 1086, not DC.

Herbaceus perennis ubique glabrus vel sparsissime tomentellus; caule tereti erecto circiter 1 m. alto striato plus minusve purpurascenti; foliis radicalibus et eis caulinis infimis petiolatis vel sessilibus amplexicaulibusque runcinato-pinnatifidis, lobis remotis, usque ad 4 dm. longis 3–18 cm. latis utrinque glabris subtus pallidioribus calloso-dentatis, summis sessilibus et auriculato-amplexicaulibus indivisis lanceolato-attenuatis; inflorescentiis terminalibus laxe paniculatis polycephalis; capitulis discoideis 10–12 mm. altis calyculatis 15–34-floris; involucri squamis plerumque 13 (8–13) lineari-lanceolatis 8–10 mm. longis acutis glabris et corollis plus minusve purpurascentibus; pappi setis albis; achæniis striatis glabris.

Specimens examined:

Mexico: State of Mexico, Désierto Viejo pres Mexico, Bourgeau, 1086 (Gray Herb. and Berlin Herb.); near Guapimalpam, coll. of 1855, Schaffner (Gray Herb.); fir woods, Sierra de las Cruces, 11 December, 1892, C. G. Pringle, 5313 (Gray Herb.); Sierra de las Cruces, altitude 3350 m., 11 February, 1899, C. G. Pringle, 7709 (Mo. Bot. Gard. Herb.); Mt. Ixtaccihuatl, altitude 2430-3350 m., C. A. Purpus, 100 (Gray Herb.); fir forests, Mt. Ixtaccihuatl, altitude 3350-3650 m., February, 1903, C. A. Purpus, 45 (Mo. Bot. Gard. Herb.). State of Vera Cruz, Las Vigas, near Jalapa, 2 December, 1903, C. G. Pringle, 11869 (Gray Herb.), forma. State of Oaxaca, without definite locality, Cuming (Gray Herb.). State of Colima, coll. of Jan. 9-Feb. 6, 1891, Dr. Edward Palmer, 1145 (Gray Herb.), distributed as "Erechthites runcinata DC."

The examination of a large suite of herbarium specimens, particularly in the light of recently acquired material, has led the writer to place a somewhat different interpretation on this species than formerly; hence, a brief description is here given and a few specimens from widely distributed exsiccati, well illustrating the species, are cited.

Senecio (§ Mulgedifolii) Coulteri Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen.

Cacalia runcinata Less. Linnæa 5: 162. 1830, not HBK.

Herbaceus perennis; caulibus erectis 3-6 dm. altis striatis paulo tomentulosis plus minusve purpurascentibus; foliis inferioribus petiolatis runcinato-pinnatifidis usque ad 3 dm. longis 1.5-6 cm. latis supra glabris subtus arachnoideo-tomentulosis inæqualiter et obtuse calloso-dentatis, foliis superioribus gradatim reductis sessilibus amplexicaulibusque; inflorescentiis terminalibus subcorymboso-cymosis; capitulis numerosis discoideis ca. 1 cm. altis brevi-calyculatis; bracteis involucri plerumque 13 lanceolatis acutis 8 mm. longis glabris et purpurascentibus; floribus disci 30-40; achæniis glabris.

Specimens examined:

Mexico: State of Vera Cruz, Real del Monte, Dr. Thomas Coulter, 429 (Gray Herb.), TYPE, C. Ehrenberg, 381 (Berlin Herb. and Gray Herb.); Mt. Orizaba, Schiede, 363 (Berlin Herb.). State of Mexico, on Nevada de Toluca, 15 October, 1903, J. N. Rose & J. N. Painter, 7940 (U. S. Nat. Herb. and Gray Herb.).

Senecio (§ Mulgedifolii) iodanthus Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32:21. 1902, nomen. Plate 12.

Herbaceus perennis; caulibus 5–9 dm. altis foliaceis striatis glabris plus minusve purpurascentibus; foliis inferioribus plerumque lyrato-pinnatifidis oblongo-lanceolatis 1.5–3 dm. longis 3.5–9 cm. latis acutis vel acuminatis sinuato-calloso-dentatis supra glabris subtus juventate arachnoideo-tomentulosis et sæpe crispo-puberulentis fere glabratis, foliis superioribus sursum gradatim reductis sessilibus amplexicaulibusque; inflorescentiis racemoso-paniculatis 2–5 dm. longis 0.3–1.2 dm. latis; capitulis 10–12 mm. altis discoideis calyculatis; bracteis involucri circiter 13 lanceolatis 8 mm. longis acutis vel obtusis penicillatis glabris vel sparse puberulentis purpurascentibus; floribus disci ca. 24; pappi setis albis quam corolla brevioribus; corollis albis vel purpurascentibus; achæniis glabris.

Specimens examined:

Mexico: State of Mexico, in pine woods, Nevada de Toluca, altitude 3000-3600 m., 26 September, 1892, C. G. Pringle, 4302 (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE. State of Morelos, Tres Marias Mts., altitude 2895 m., 5 November, 1903, C. G. Pringle, 11498 (Gray Herb.).

This species is closely related to S. Coulteri Greenm. but differs in having a smooth and more leafy stem, nearly glabrous leaves, and distinctly racemose-paniculate inflorescence.

Senecio purpurascens Klatt, Leopoldina, Heft 24, p. 126. 1888.

Var. fossanervius Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen.

Formæ typicæ habitu simili; foliis inferioribus petiolatis, petiolo incluso, usque ad 11 cm. longis 1.5 cm. latis sinuato-dentatis vel ad basin sublyratis supra glabris fossanerviis subtus tomentellis et in nerviis pilosis; involucri squamis fere glabris.

Specimen examined:

Mexico: without definite locality, E. W. Nelson, 1308 in part (U. S. Nat. Herb., fragments in Gray Herb.), TYPE.

Senecio (§ Suffruticosi) carnerensis Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902, nomen.

Perennis basi suffrutescens ubique plus minusve lignescens; caule tereti erecto simplici vel ramoso; foliis indivisis petiolatis vel sessilibus lanceolatis vel oblanceolatis 1.5-5 cm. longis usque ad 1 cm. latis acutis denticulatis juventate utrinque tomentosis

supra plus minusve glabratis subtus persistenter albo-tomentosis, superioribus subauriculatis; inflorescentiis terminalibus paucicapitatis; capitulis ca. 1 cm. altis brevi-calyculatis radiatis; bracteis involucri plerumque 13 anguste lanceolatis apice atratis acutis glabris vel parce tomentulosis; flosculis liguliferis plerumque 8, ligulis flavis 4-nerviis; floribus disci 30–40 achæniis sursum brevi-sericeo pubescentibus.

Specimen examined:

Mexico: State of Coahuila, mountains, Carneros Pass, altitude 3050 m., 8 September, 1889, C. G. Pringle, 2857 (Gray Herb., photograph in Mo. Bot. Gard. Herb.), TYPE.

This species was originally referred to S. longilobus Benth., but it is more closely allied to S. stoechadiformis DC. and S. Picridis Schauer; it is readily separated from both these species by having fewer involucral bracts, short, appressed and black-tipped bracteoles suggesting those of S. vulgaris L.

Senecio (§ Suffruticosi) filicifolius Greenm. Monogr. Senecio, pt. 1, 25. 1901; in Engl. Bot. Jahrb. 32: 21. 1902; Contr. U. S. Nat. Herb. 16: 19. 1912, nomen.

Herbaceus perennis (?) erectus ramosus 1.5-4 dm. altus ubique glabrus; caule tereti ad basin plus minusve lignescenti; ramis ramulisque striatis stramineis; foliis sessilibus vel subalato-petiolatis pectinato-pinnatifidis 1.5-8 cm. longis 1-6 cm. latis; segmentis linearis attenuatis acutis; inflorescentiis subcorymbosocymosis oligocephalis; capitulis ca. 12 mm. altis ligulatis; involucris campanulatis calyculatis; involucris squamis plerumque 21 bracteolis calyculatis duplo longioribus lineari-lanceolatis acutis glabris vel juventate parce tomentulos's mox glabratis; flosculis liguliferis ca. 12, ligulis flavis; floribus disci 50-60; pappi setis albis; achæniis sursum sericeo-hispidulis.

Specimens examined:

Arizona: Valley of the Santa Cruz River, 11 May, 1881, C. G. Pringle, 316 (Gray Herb.), TYPE; Tucson, 12 March, 1892, J. W. Toumey, 708 (Gray Herb.); Tempe, coll. of 1892, Ganong & Blaschka (Gray Herb.); Hart's Ranch, 17 miles south of Tucson, 11 April, 1903, J. J. Thornber, 436 (Mo. Bot. Gard. Herb.); Ft. Huachuca, coll. of 1894, Maj. T. E. Wilcox (Mo. Bot. Gard. Herb.); open cañons, San Francisco Mts., April, 1887, H. H. Rusby, 214 in part (Mo. Bot. Gard. Herb.).

Mexico: Sandy plains near Altar, State of Sonora, 4 April, 1884, C. G. Pringle (Gray Herb.).

This species has been hitherto included with S. Douglasii DC. from which it differs in being essentially glabrous throughout, in having usually more numerous and shorter lateral leaf-segments, fewer, shorter, and less conspicuous calyculate bracteoles.

Senecio (§ Suffruticosi) teliformis Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

Herbaceus perennis: caule erecto tereti superne striato stramineo floccoso-tomentoso plus minusve glabrato; foliis supremis sessilibus lanceolato-attenuatis 3-6 cm. longis ad basin ampliatis usque ad 1.5 cm. latitudine semiamplexicaulibusque supra juventate floccoso-tomentulosis plus minusve glabratis subtus persistenter albo-tomentosis, margine dentatis vel denticulatis revolutisque; foliis inferioribus ignotis; inflorescentiis terminalibus corymboso-cymosis multicapitatis bracteatis floccoso-tomentulosis: capitulis 8-10 mm. altis radiatis calvculatis, bracteolis calvculatis lineari-attenuatis conspicuis subflaccidis floccoso-pubescentibus; involucri bracteis plerumque 21 linearilanceolatis 5-6 mm, longis acutis glabris penicillatis; flosculis liguliferis sæpius 8, ligulis oblongis 5-6 mm. longis flavis; floribus disci ca. 40 quam bracteis involucri longioribus, pappi setis albis; achæniis sursum adpresso-sericeo-pubescentibus maturitate 3 mm. longis.

Specimen examined:

Mexico: State of Oaxaca, mountains of Telixtlahuaca, altitude 2500 m., 10 December, 1894, Rev. Lucius C. Smith, 367 (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE.

Although only the upper part of the plant is at present known to the writer, nevertheless it evidently belongs to the section Suffruticosi and appears to be most closely related to S. Picridis Schauer and S. alvarezensis Greenm. From the former it differs by the usually broader base of the upper stem leaves, more numerous heads and conspicuous bracteoles, while from the latter it is readily separated on foliar characters alone.

Senecio (§ Palmatinervii) albonervius Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

Arborescens 2-4 m. altus; caule tereti primo albo-tomentuloso maturitate glabrato et cortice brunneo tecto; foliis petiolatis basi palmatinerviis late ovatis 3-5 cm. longis latisque sinuato-5-11-lobatis remote calloso-mucro-denticulatis basi cordatis juventate utrinque tomentulosis plus minusve glabratis supra in nerviis persistenter albo-tomentulosis, petiolis plerumque 3-10 (usque ad 14 cm.) longis; inflorescentiis terminalibus paniculatis multicapitatis; capitulis 10-12 mm. altis radiatis; involucris anguste campanulatis vel subcylindricis brevicalyculatis; involucri squamis circiter 8 lineari-lanceolatis vel oblongis obtusis 5-6 mm. longis glabris vel parce tomentulosis; flosculis liguliferis plerumque 5, ligulis 5-7 mm. longis flavis 4-nervatis, pappi setis tubo corollæ longioribus; floribus disci 8-10; achæniis glabris.

Specimens examined:

Mexico: State of Mexico, Valley of Temascaltepec, April, 1831, Schiede (Berlin Herb. and Gray Herb.), TYPE; open woods, Ixtaccihuatl, altitude 2430–3350 m., March-July, 1903, C. A. Purpus, 201 (Gray Herb. and Mo. Bot. Gard. Herb.). State of Vera Cruz, Mineral del Monte, Ehrenberg, 324 (Berlin Herb. and Gray Herb.). State of Morelos, Sierra de Tres Marias, altitude 3050 m., 15 April, 1904, C. G. Pringle, 8903 (Gray Herb. and Mo. Bot. Gard. Herb.). State of Michoacan, north slope of Mt. Tancitaro, altitude 2280–3200 m., 24 February, 1903, E. W. Nelson, 6904 (U. S. Nat. Herb. and Gray Herb.).

The broadly ovate, shallowly sinuate-lobed leaves with persistent white tomentum on the veins of the upper leaf-surface, together with a terminal many-headed panicle and yellow ray-flowers, render this species distinct and easily recognized among all those of the palmately veined section to which it belongs.

Senecio angulifolius DC., var. ingens, var. nov.

Habitu et foliis formæ typicæ; inflorescentiis compactis pauci- vel multi-capitatis, bracteis bracteolisque perconspicuis; capitulis 1.5–2 cm. altis 40–45-floris radiatis vel discoideis.

Specimens examined:

Mexico: Mt. Ixtaccihuatl, above timber line, March-July, 1903, C. A. Purpus, 193 (Mo. Bot. Gard. Herb.), TYPE; rocky slopes, Mt. Ixtaccihuatl, altitude 5790-6090 m.,

November, 1905, C. A. Purpus, 1517 (Mo. Bot. Gard. Herb.). State of Puebla, Mt. Orizaba, near Chalchicomula, 25 February, 1892, Jared G. Smith, 473 (Mo. Bot. Gard. Herb.).

On account of the conspicuous more or less foliaceous bracts of the inflorescence S. angulifolius DC. is a very characteristic  $\varepsilon_1$  ecies and is almost always recognized without difficulty. There is, however, a considerable variation in the size of the heads and in the number of flowers of the disk, as well as in the degree of development of the ray-flowers. In fact the latter may be well developed, more or less reduced, or entirely absent. The extremely large headed form, which is well exemplified by the specimens cited above, seems well worthy of varietal recognition. Doctor Purpus's No. 1517 is somewhat intermediate between the species and the variety.

Senecio (§ Palmatinervii) brachyanthus Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

Verisimiliter frutex; caule tereti cortice brunneo tecto juventate hirtello-puberulento glabrato; foliis longipetiolatis subpeltatis palmatinerviis suborbicularis circiter 7-lobatis membranaceis utrinque parce hirtellis subtus pallidioribus mucrodenticulatis, petiolis usque ad 13 cm. longis minute puberulentis; inflorescentiis terminalibus subglanduloso-hirtellis; capitulis subcylindricis 10–12 mm. altis heterogamis; involucri bracteis 8 lanceolatis 8–10 mm. longis acutis vel obtusis plus minusve purpurascentibus extus subglanduloso-hirtellis; flosculis femineis 5 multum reductis, ligula nulla, tubo gracili squamis involucri breviore; floribus disci 8–10; pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: State of Guerrero, between Ayusinapa and Petatlan, altitude 1540-2155 m., E. W. Nelson, 2137 (Gray Herb. and U. S. Nat. Herb.), TYPE.

The leaves and reduced ray-flowers of this species are similar to those of *S. cordovensis* Hemsl., but the character of the involucre indicates a closer relationship with *S. chapalensis* Watson.

Senecio (§ Palmatinervii) chapalensis Watson, Proc. Am. Acad. 25: 155. 1890.

Var. areolatus Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

A forma typica recedit foliis utrinque glabratis subtus areolatis, petiolis usque ad 15 cm. longis plus minusve purpurascentibus; flosculis liguliferis granditer reductis.

Specimen examined:

Mexico: State of Morelos, on shaded bluffs of a wet canyon above Cuernavaca, altitude 1980 m., 15 February, 1899, C. G. Pringle, 8010 (Gray Herb. and Mo. Bot. Gard. Herb.), TYPE.

Senecio (§ Palmatinervii) Chrismarii Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

Frutex; caule primo parce pubescenti maturitate glabro; foliis petiolatis palmatinerviis circumscriptione triangulariovatis 7–10 cm. longis 5–8 cm. latis hastatis 3–5-lobatis ciliatis mucro-denticulatisque granditer cordatis supra sparse hirtellopuberulentis subtus glabris vel in nervis puberulentis, lobiis mucronato-acutis; petiolis gracilibus 4–9 cm. longis parce hirtellis vel glabris; inflorescentiis terminalibus laxe paniculatis paucicapitatis dense glanduloso-puberulentis, pedunculis gracilibus remote bracteatis; capitulis 1.2–1.5 cm. altis discoideis paucicalyculatis; involucri squamis sæpius 8 lanceolato-oblongis ca. 1 cm. longis acutis penicillatis extrinsecus hirtello-puberulentis plus minusve purpurascentibus interioribus scariosomarginatis; floribus disci plerumque 20 involucri bracteis longioribus; pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: without definite locality, Chrismar (Berlin Herb., tracing and fragments in Gray Herb.), TYPE.

The affinity of this species is with S. hederæfolius Hemsl., S. anisophyllus Klatt, and S. alienus Robinson & Seaton. From the first two it differs in having deeply cordate leaves with more or less reflexed lateral lobes, and from the last it is readily separated by the deeply cordate leaves and absence of peltation.

Senecio (§ Palmatinervii) hypomalacus Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen. Plate 10.

Frutex erectus; caule tereti primo dense sordido-puberulento, sæpissime lenticellis intermixtis, maturitate cortice brunneo tecto; foliis petiolatis vel supremis sessilibus circumscriptione ovato-rotundatis vel ovato-oblongis palmato-3-5-nerviis distincte 5-11-lobatis supra crebe crispo-hirtellis subtus lanato-tomentosis basi cordatis vel subtruncatis, margine sinuatis calloso-denticulatis ciliatis; petiolis usque ad 6 cm. longis; inflorescentiis terminalibus paniculatis polycephalis subglanduloso-hirtellis; capitulis 10-12 mm. altis parce calyculatis radiatis; bracteis involucri plerumque 8 (non-nunquam 7) oblongis vel subobovatis 5-6 mm. longis obtusis vel acutis extus crebe subglanduloso-hirtellis, interioribus late scarioso-marginatis; flosculis femineis liguliferis, ligulis anguste oblongis 5-6 mm. longis flavis; floribus disci circiter 10 (7-13) quam involucrum bis tanto fere longioribus; pappi setis albis; achæniis glabris.

Specimens examined:

Mexico: State of Oaxaca, mountains of Telixtlahuaca, altitude 2375 m., 10 December, 1894, Rev. Lucius C. Smith, 368 (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE; Sierra de San Felipe, altitude 2130-2440 m., 17 November, 1894, Charles L. Smith, 210 (Mo. Bot. Gard. Herb.); Cerro de San Felipe, altitude 1900 m., 25 September, 1895, C. Conzatti, 119 (Gray Herb.).

This species is related to S. oaxacanus Hemsl., but differs from it in having distinctly lobed leaves which are thicker in texture, densely subglandular-hirtellous above and soft tomentose beneath; moreover, the leaf-margin of S. hypomalacus is markedly sinuate and the lobes show a tendency to become again lobate. C. and E. Seler's No. 1581 from Tillantongo, which has been referred to S. oaxacanus Hemsl., is somewhat intermediate between the two species, but it has the leaf-outline and thinner texture of Mr. Hemsley's species.

Senecio (§ Palmatinervii) Kerberi Greenm. Monogr. Senecio, pt. 1, 26. 1901; in Engl. Bot. Jahrb. 32: 22. 1902, nomen.

Herbaceus robustus perennis usque ad 3m. altus; caule tereti erecto glabro vel parce tomentuloso; foliis petiolatis palmato-5-7-nerviis ovato-oblongis 5-10 cm. longis 5-8 cm. latis 5-7-lobatis carnoso-denticulatis reticulato-venosis supra sparse hirtellis subtus subarachnoideo-tomentulosis, lobis obtusis vel

subrotundatis et mucronato-acutis; petiolis 2-2.5 cm. longis; inflorescentiis terminalibus paniculatis multi-capitatis pubescentibus, pedunculis minute bracteatis; capitulis 7-8 mm. altis radiatis; involucris campanulatis minute calyculatis fere glabris; involucri squamis 13 lineari-lanceolatis vel lanceolato-oblongis 4.5-5 mm. longis acutis glabris; flosculis femineis 5 liguliferis, ligulis oblongis 4-5 mm. longis flavis; floribus disci ca. 14, pappi setis albis; achæniis glabris.

Specimen examined:

Mexico: "Tromptero, Mesa del Arrero," 21 November, 1880, Kerber, 94 (Berlin Herb., fragments and tracing in Gray Herb.), TYPE.

This species is known at present from a single specimen in the Royal Botanical Museum of Berlin. From this specimen the writer was permitted, as in a number of other cases, while making a study of the genus several years ago, to make a tracing and take fragments for the Gray Herbarium of Harvard University. The species is related to S. Hartwegi Benth. and S. reglensis Greenm., but from these and from other species of the section Palmatinervii to which it belongs, it is readily distinguished by the somewhat elongated more or less fan-shaped and bluntly lobed leaves.

Senecio (§ Palmatinervii) velatus, sp. nov. Plate 13. Frutex; caule tereti carnoso ramoso ad apicem sordido-tomentoso cetero glabro in sicco cortice brunneo tecto; foliis petiolatis palmato-7-nerviis circumscriptione ovato-rotundatis ca. 10 cm. longis latisque angulato-7-9-lobatis membranaceis integris juventate utrinque plus minusve albo-tomentosis subtus persistenter arachnoideo-tomentulosis, lobis triangulari-ovatis mucronato-acutis; petiolis ca. 8 cm. longis floccoso-pubescentibus; inflorescentiis terminalibus dense cymoso-corymbosis minute bracteatis multicapitatis glabris vel in axillis ramulorum floccosotomentulosis; capitulis ca. 1.5 cm. altis radiatis; involucri subcylindrici squamis sæpius 8 lanceolato-linearis vel lanceolatooblongis 7-10 mm. longis acutis vel obtusis; flosculis liguliferis 3-5, ligulis anguste oblongis ca. 1 cm. longis; floribus disci 6-7, pappi setis albis; achæniis glabris striatis.

Specimen examined:

Mexico: State of Jalisco, on bluffs of barranca, near Guadalajara, 20 May, 1891, C. G. Pringle, 5160 (Gray Herb., photograph and fragments in Mo. Bot. Gard. Herb.), TYPE.

The writer has withheld publication of this species for several years with the hope that additional material might be secured. Mr. Pringle's specimen, from which the above description is drawn, is in the Gray Herbarium and consists of a terminal portion of a flowering stem and two detached leaves. In stem and inflorescence characters it corresponds very well with typical specimens of S. præcox DC. except that the terminal portion of the stem and branches are covered with a tawny pubescence, not glabrous as is usually the case with the DeCandollean species. On account of the similarity of stem and inflorescence and because of the detached leaves the plant has been referred doubtfully to the peculiarly characteristic and well known S. præcox DC.

The extreme care with which Mr. Pringle prepared his plant material and the fact that the leaves on the specimen under consideration, although detached from the stem, accord with the type of foliage of the section *Palmatinervii* lead me to believe that we have to deal in the present case with an unrecorded species related to but distinct from S. præcox DC., and in all probability one of limited geographical distribution.

Senecio Klattii, nom. nov.

S. roseus Klatt, Ann. k. k. Naturhist. Hofmus. Wien 9: 366. 1894, not. S. roseus Schz. Bip. in Flora 28: 498. 1845.

## EXPLANATION OF PLATE

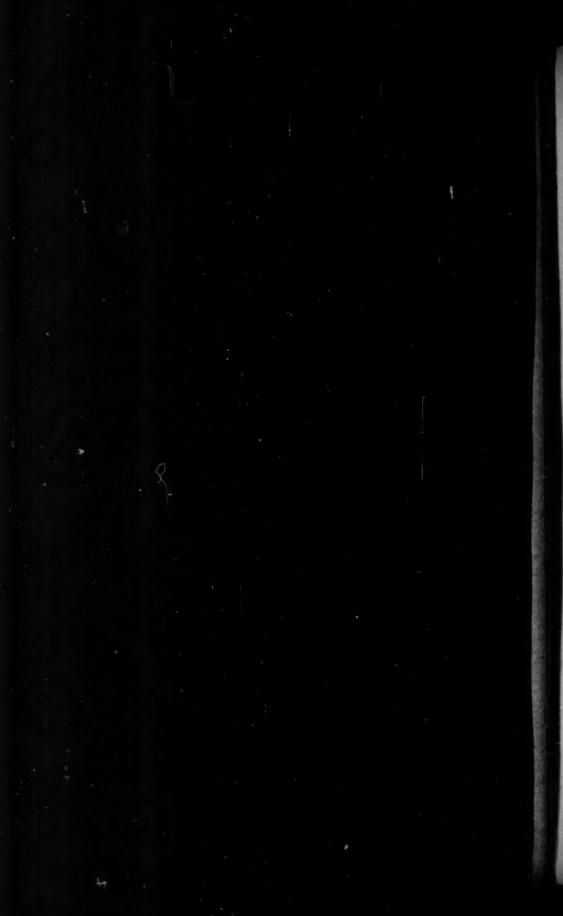
### PLATE 10

Senecio hypomalacus Greenm.

#### Mexico

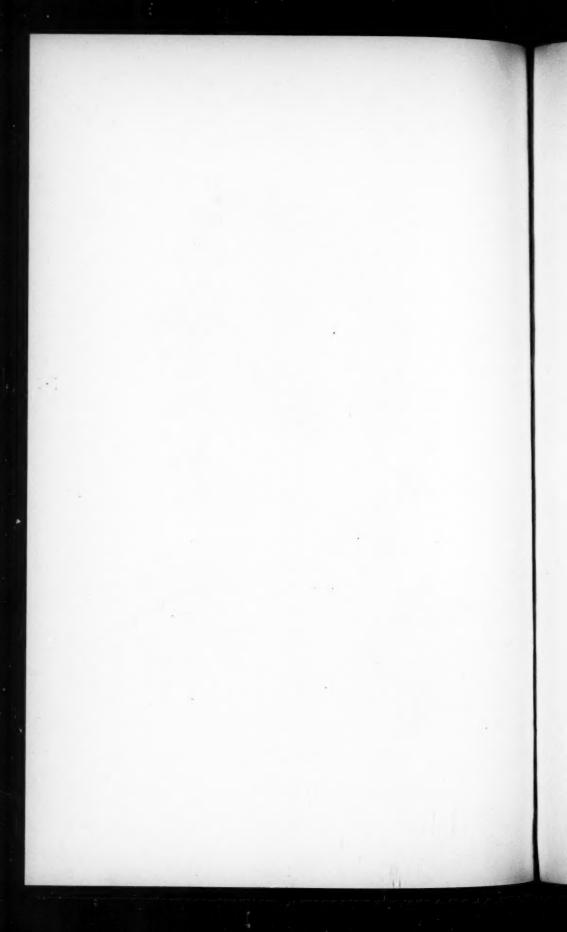
From the type specimen, Rev. Lucius C. Smith No. 368, in the Gray Herbarium of Harvard University.

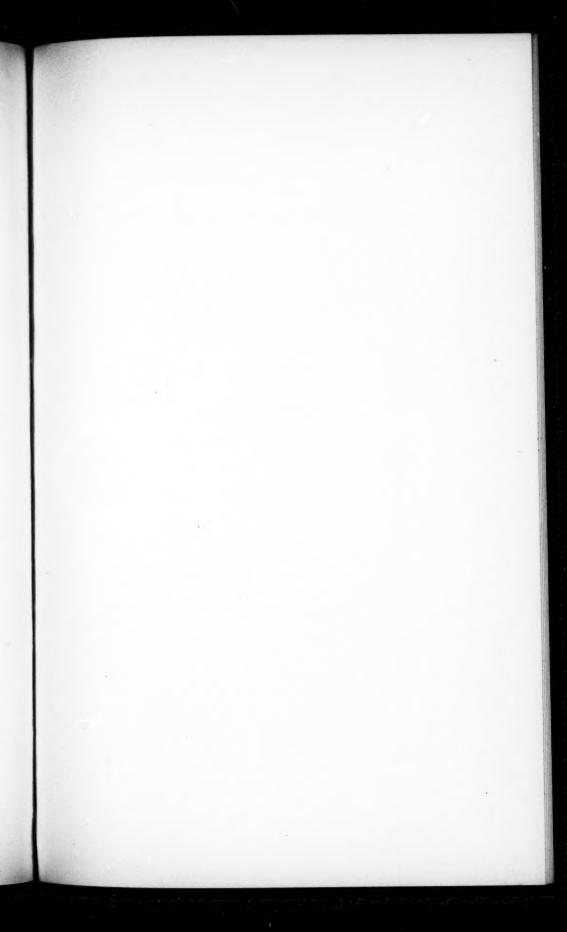






GREENMAN-NORTH AMERICAN SENECIONEAE





## EXPLANATION OF PLATE

## PLATE 11

Senecio oreopolus Greenm.

#### California

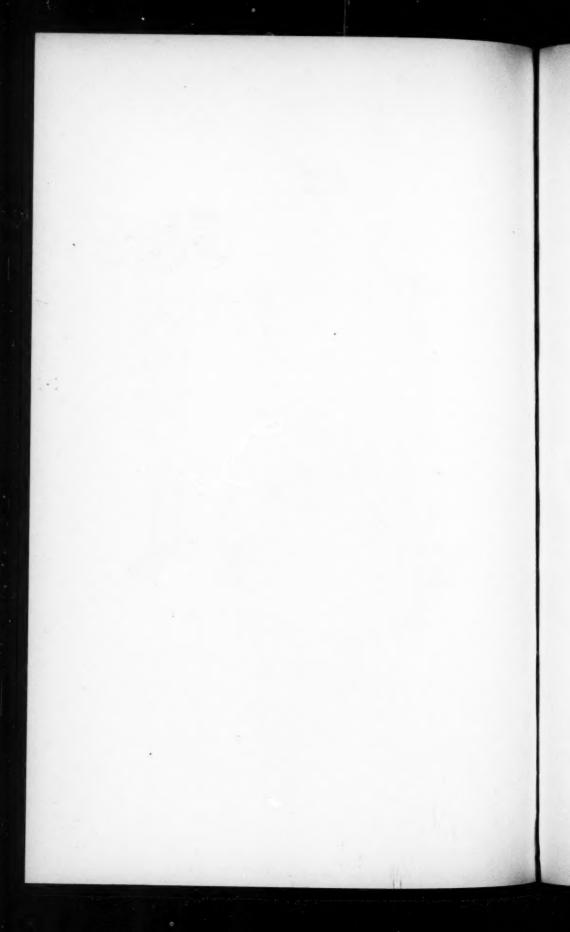
From the type specimen, Hall and Babcock No. 5526, in the Gray Herbarium of Harvard University.

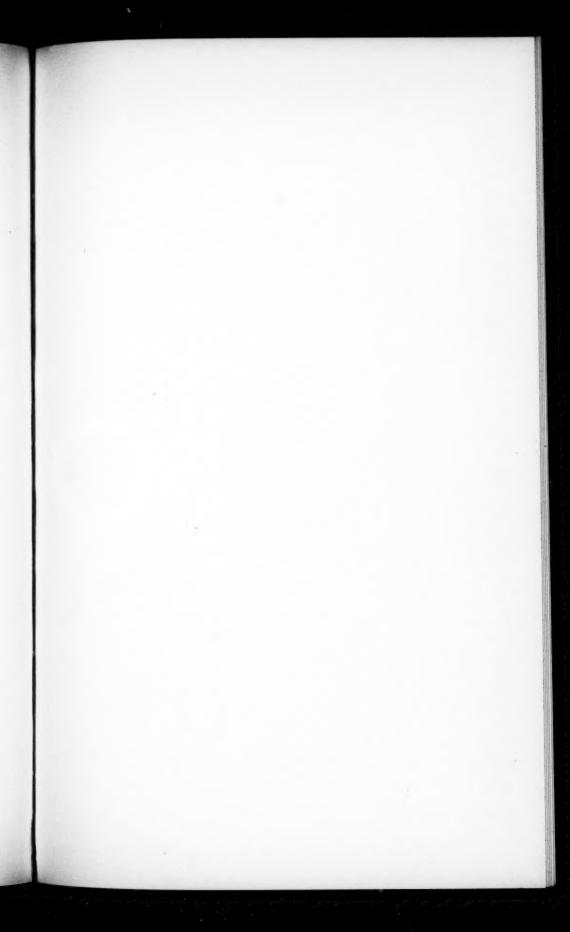






GREENMAN - NORTH AMERICAN SENECIONEAE





### EXPLANATION OF PLATE

#### PLATE 12

Senecio iodanthus Greenm. Mexico

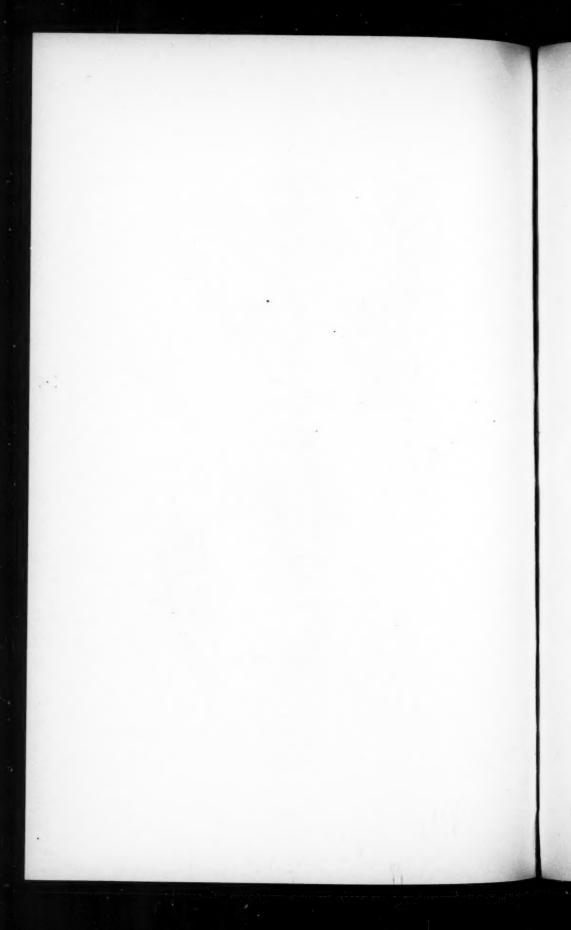
From the type specimen, Pringle No. 4302, in the Gray Herbarium of Harvard University.

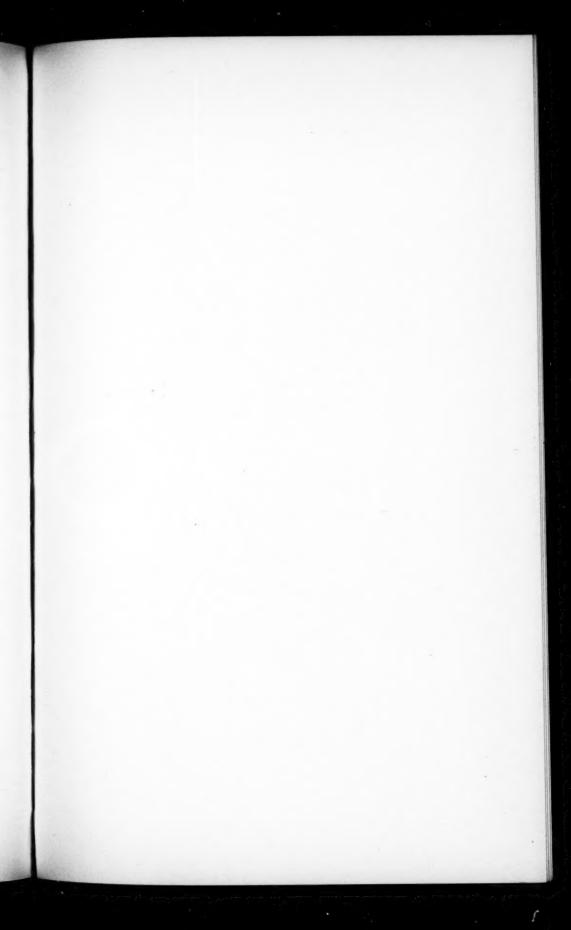






GREENMAN - NORTH AMERICAN SENECIONEAE





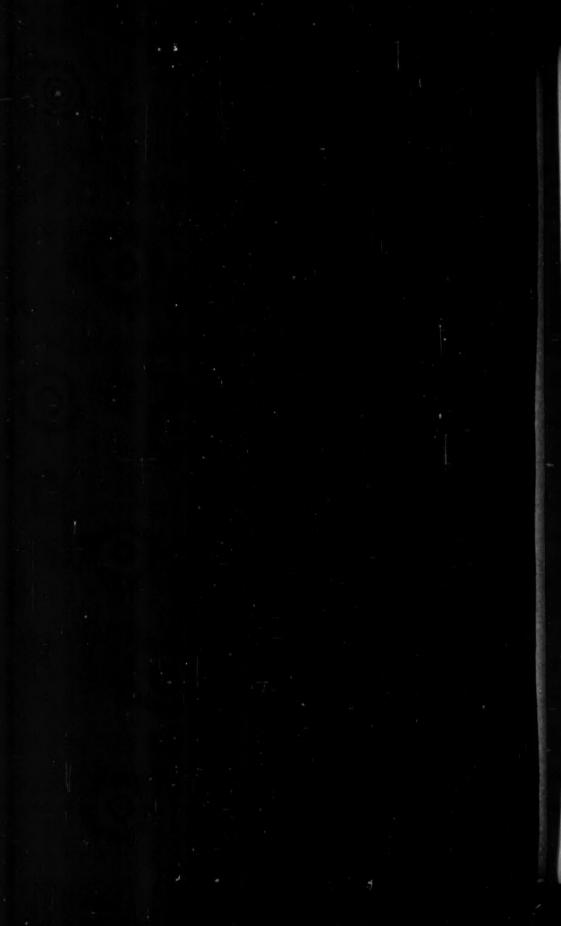
## EXPLANATION OF PLATE

## PLATE 13

Senecio velatus Greenm. Mexico

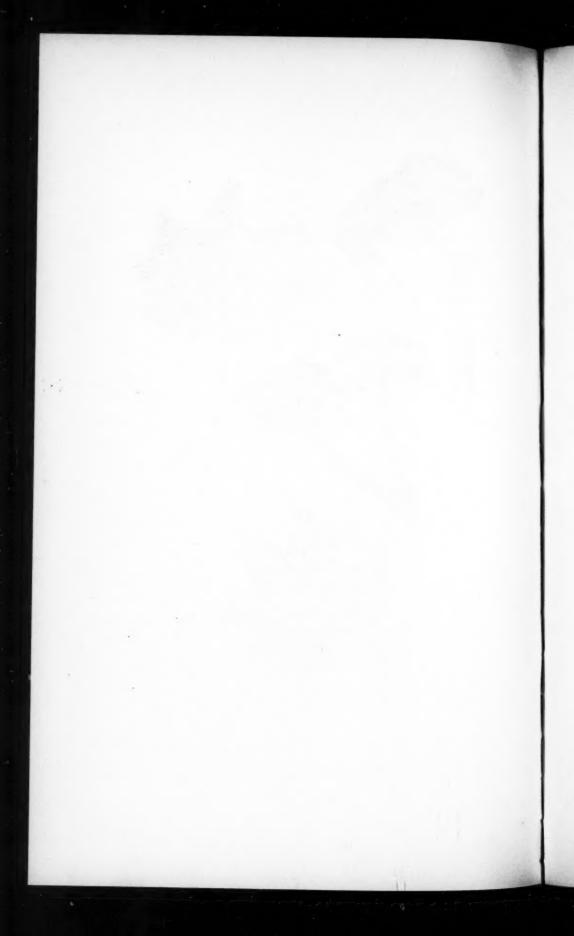
From the type specimen, Pringle No. 5160, in the Gray Herbarium of Harvard University.

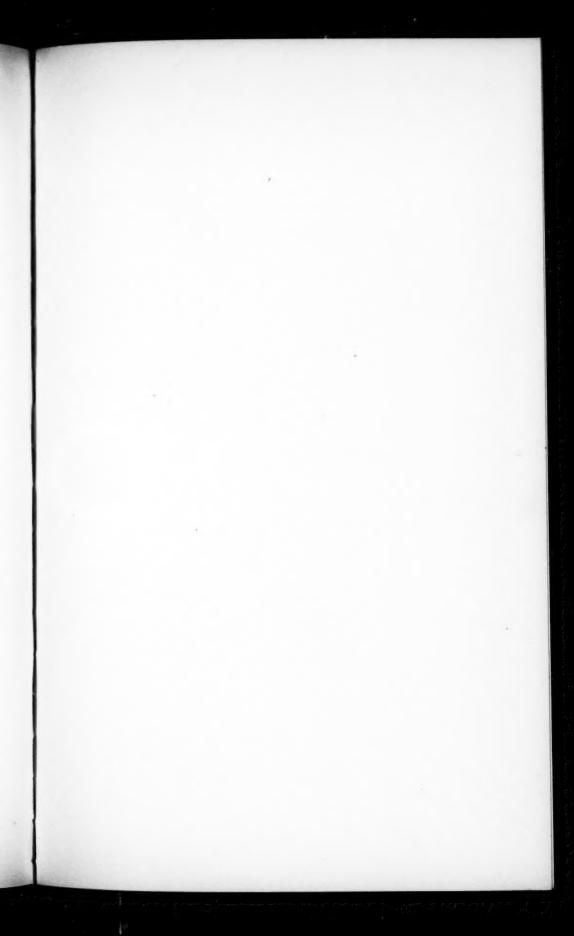






GREENMAN - NORTH AMERICAN SENECIONEAE





# EXPLANATION OF PLATE

## PLATE 14

Senecio subauriculatus Greenm. Mexico

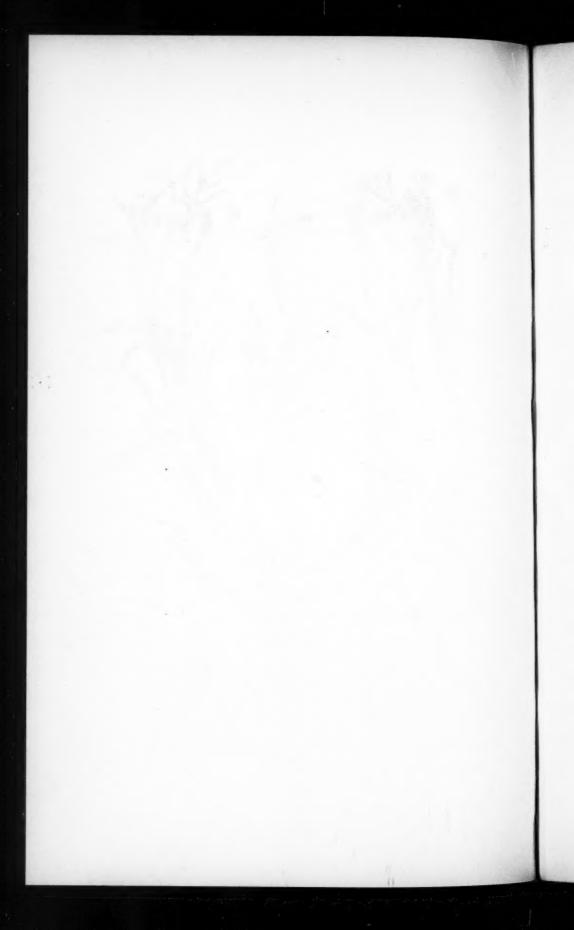
From the type specimen, E. W. Nelson No. 2526, in the Gray Herbarium of Harvard University.







GREENMAN - NORTH AMERICAN SENECIONEAE



# A STUDY OF THE PHYSIOLOGICAL RELATIONS OF SCLEROTINIA CINEREA (BON.) SCHRÖTER

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#### INTRODUCTION

This paper reports the results of an experimental study regarding certain physiological activities of the brown-rot fungus of stone fruits. The investigation concerns itself primarily with the conditions influencing the penetration and infection of green and ripe fruits by the fungus in question, the action of the parasite on the host cell, and the secretion of the enzymes which act upon the cellulose and pectic substances of the host. The work was undertaken with the hope of throwing some further light upon the factors concerned in fungous parasitism. Our present conception of this subject is based upon fragmentary and, in some respects, contradictory evidence. However, each year there are acquired new facts, or new applications of known facts, bearing upon this exceedingly involved and complex question. An examination into the history of investigations concerning the interaction of host and parasite shows that the study of this subject dates back to the work of the pioneers in plant pathology; modern methods and recent discoveries have, however, given an added impetus to research along this line.

Progress in combating fungous diseases depends not only upon a familiarity with the life history of the parasite, but more especially upon an intimate knowledge of the metabolism of the parasite and the nature of the changes which it induces in the host. Indeed, many of our recommendations for controlling parasitic diseases of plants will perhaps be modified when a more exact knowledge of the interrelations of host and parasite is gained. Furthermore, a more intimate knowledge of the physiological aspects of plant pathology will undoubtedly throw much light on the question of immunity and susceptibility.

We should, of course, like to know more about the factors favoring or inhibiting parasitic action, as well as the conditions

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which influence the infection and the penetration of parasitic fungi. It would also be interesting to know why some fungi are so virulent and rapid in their destructive action on the host; for instance, it would be instructive to know whether it is due to the secretion of an enzyme, or a toxic substance (e. g., some acid), or to the disturbance of the osmotic relations of the host cells, or to some other perhaps unknown factor. For a study of some of these problems the writer has chosen as the organism Sclerotinia cinerea (Bon.) Schröter, the fungus causing the brown rot of stone fruits. This form is particularly suitable for the purpose since it is a virulent parasite, yet grows well as a saprophyte—readily lending itself to cultivation in the laboratory.

## HISTORICAL REVIEW

Space will permit only a brief review of some of the more important papers dealing with certain aspects of this subject. Much of the literature that is indirectly concerned with the problem, or that is fully reviewed or superseded by subsequent publications, will not be discussed here.

In the period from 1858 to 1878 little experimental evidence appeared concerning the nature of the action of fungous parasites, although several writers make mention of the penetration of host cells by fungous hyphae. Penetration was then frequently spoken of as merely a process of boring through ("durchbohrung") the host tissue, Kühn (34), as early as 1858, mentioning this fact in a discussion of the potato-blight fungus. A few years later, in 1863, de Bary (1) speaks of the penetration of the host by Peronospora, and further makes mention of this fact in connection with his work on the rusts (2); again in his work 'Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten' (3) he discusses the penetration of the host, but says he has no knowledge of the force that causes this boring into the host tissue.

Hartig (26), in his early work on wood-destroying fungi, as well as in his later investigations, emphasizes the fact that fungi are able to destroy cellulose. By a microscopical study of diseased wood he found that the properties of the latter are very materially changed by the fungus; he did not, however, attempt to isolate an enzyme.

De Bary (4), in 1886, gives us the first important contribution to our knowledge concerning the action of parasites on host This author, in his epoch-making research on the fungus now known as Sclerolinia libertiana, reports that the organism secretes a substance that discolors, plasmolyzes, and finally This toxic secretion penetrates the host kills the host cells. cells in advance of the fungus, killing them before they are actually pierced by the fungous filaments. De Bary was able to isolate this toxic substance, which he considered as probably an enzyme, and found that it would cause an injury to the host tissue similar to that produced by an attack of the fungus itself. He holds that the fungus will not grow on living tissues. for it attacks only through a wound and kills the cells in advance of itself, thus not actually growing upon the living tissue. The product resulting from the disintegration of the cell wall of the host was thought to be a sugar that served as food for the fungus. In this connection de Barv also mentions finding oxalic acid encrusting the older fungous filaments.

The next important paper on the interaction of host and parasite was that of Marshall Ward (51) published just two years after de Bary's work and concerning itself with a species of Botrytis causing a lily disease. In this excellent piece of work the author showed that the fungous hyphae on coming in contact with such solid substances as sections of a lily bulb, or even a cover glass, secrete from the tips drops of a substance that has a very peculiar effect on the host cell. He found that a water extract of this secretion when applied to sections of a lily bulb will cause the cell walls to swell and to assume an abnormal appearance; the middle lamella is first dissolved and finally the entire cell wall is disorganized. Ward does not consider that this toxic secretion is stimulated by starvation.

Several investigators have held that the penetration of many fungi is due to chemotropism, i. e., that penetration of the fungous hyphae is due to some stimulus which the constituents diffusing slowly from within the host cells exert. Büsgen (16), Miyoshi (39), Behrens (6), Schmidt (44), and others have adhered to the view that chemotropism is important, but more recent work, such as that of Fulton (25), does not uphold the theory.

Behrens (6) investigated some of the physiological relations of saprophytes in comparison with parasites, using Mucor stolonifer, Penicillium sp., Botrytis cinerea, and Oidium (=Sclerotinia1) fructigenum. This author holds that Sclerotinia does not produce a cellulose-dissolving enzyme, and that the fungus merely forces its way through the host tissue by a purely mechanical force, or that, in some cases, it splits the middle lamella but does not dissolve it. In the case of the other fungi mentioned above he believes that an enzyme is secreted which dissolves the middle lamella. The cause of the injury due to Sclerotinia. he holds, is not that the cellulose walls or the pectin of the middle lamella is dissolved, but that the turgor and the osmotic relations of the penetrated cells are materially modified. According to this author some substance diffuses through the walls and stimulates the fungus to bore through or between the He demonstrated in Botrutis and Penicillium, morecell walls. over, a thermo-stable toxic body which disintegrated the host cells, and believes that these fungi secrete a pectin-dissolving enzyme which is different from that which acts upon cellulose.

Nordhausen (40), at about the same time, made similar studies on Botrytis cinerea and comes to similar conclusions. He finds that the enzyme does not cause a strong swelling of either the middle lamella or the cellulose cell walls, the action in this respect being more like that of de Bary's Sclerotinia. Smith (46) studied the parasitism of Botrytis cinerea, but in certain particulars did not get the same results as de Bary and Ward. Like them he finds that the parasite secretes some soluble substance that penetrates and kills the living cells in advance of the fungous filaments, but unlike Ward he could detect no swelling of the cell wall. Smith believes that this toxic substance is not an enzyme, for boiling does not inactivate it, but thinks that it is perhaps oxalic acid, since this substance is always present in the cultures and amounts in some cases to as much as two per cent. The analytical methods whereby the oxalic acid was determined, unfortunately, are not given.

Schellenberg (43) investigated the action of several saprophytic and parasitic fungi on hemicelluloses from a number of

<sup>&</sup>lt;sup>1</sup> Wehmer, C. Ber. d. deut. bot. Ges. 16: 298-307. 1898; Saccardo, Syll. Fung. 4: 34. 1886.

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He claims that these fungi act differently different sources. toward different celluloses, dissolving some and having no effect The nature of the penetration and the action of certain parasites on the host tissue were also studied. was no case in which Botrytis dissolved true cellulose. but it readily dissolved the hemicellulose part of the cell, leaving the cellulose intact. According to this author, therefore, the penetration and dissolving action of such parasites as Botrytis vulgaris is due to their ability to dissolve hemicelluloses. considers that the middle lamella is largely composed of hemicelluloses or closely allied substances. According to this view, therefore, organisms that dissolve the middle lamella are essentially hemicellulose-dissolving forms. As a result of his studies on Sclerotinia fructigena and S. cinerea, Schellenberg finds a different action on different fruits, but in no case does he report a splitting of the cells along the line of the middle lamella, as some previous investigators have reported. He believes that there is a slight dissolving action on that part of the cell wall which is in immediate contact with the fungous filament, but that the rest of the cell wall remains intact. In the twigs also he finds that the fungus dissolves the hemicellulose and leaves the true cellulose unacted upon.

An extensive literature has developed concerning the enzymes of importance in the nutrition of fungi, but since these investigations either deal with saprophytes, or are only indirectly concerned with the work to be reported in this paper, it will be unnecessary to do more than mention some of the papers here. Among the more important contributors may be mentioned Ward (50, 52), who was the first to use pure cultures of a wooddestroying fungus (Stereum), Biffen (9), who studied the biology of Bulgaria polymorpha, Bourquelot and Hérissey (13), who investigated the enzymes in sporophores of Polyporus sulphureus, Czapek (18), who made his investigations with natural infections of Merulius lacrymans and with other fungi, Kohnstamm (33), who worked on some species of Merulius, Buller (14, 15), who investigated sporophores of Polyporus squamosus, Van Iterson (28), who developed methods for isolating cellulose-dissolving bacteria and fungi, and Dox (19), who investigated the enzyme action of species of Penicillium and Aspergillus. It is interesting to note that although we have every reason to believe that cytase is present in timber-decay organisms yet its presence has been demonstrated only indirectly by cytological methods. It is true, however, that many of the investigators mentioned above who found no cytase used the sporophores in their experiments and not the mycelium.

The status of the subject of the enzymes concerned in the metabolism of parasitic fungi is given in Reed's recent publication (42), which concerns itself with the enzymes produced by the parasitic fungus Glomerella rufomaculans. This author has proved that the parasite produces many of the enzymes that had previously been reported for saprophytes, and by quantitative methods has demonstrated different enzymes acting on the several classes of nutritive substances, such as carbohydrates, glucosides, fats, and proteins. He did not, however, investigate the cytolytic activity of the fungus but states that the nature of the diseased host would indicate that cytase very probably is not produced by this fungus. Peltier (41), as a result of his investigations with Botrytis Fuckeliana, finds that the host cells are killed in advance of the fungous penetration, and that the parasite secretes a thermo-stable toxic substance, but, unlike Smith, finds no oxalic acid. The method of testing for oxalic acid unfortunately is not given.

The action of bacteria on cellulose and other plant products has been extensively studied by a number of investigators, but for the purpose at hand it will suffice to cite some of the more recent publications in which the earlier literature is reviewed. The work of Jones (29, 30), which gives a good resumé of the early work on this subject, is reviewed below under the discussion of pectin.

McBeth and Scales (38) report that a number of bacteria and fungi hydrolyze cellulose and claim that filamentous fungi play a very important rôle in the destruction of cellulose in soils. The cellulose-destroying fungi, according to these authors, act differently toward different kinds of cellulose, but their experiments do not seem to support this conclusion. Kellerman and McBeth (32) have also contributed to our knowledge of the cytolytic activity of fungi. Kellerman (31) has employed a method

by which it is demonstrated that cytase diffuses in agar considerably beyond the region of hyphal penetration, and that a portion of the agar containing the enzyme dissolves cellulose in a manner similar to that of the fungus itself.

The organism employed in my work was isolated from an infected plum twig, at Madison, Wisconsin. The original cultures were taken from a single colony in a Petri dish, this procedure giving reasonable assurance that I was working with a single strain of the organism. Regarding the systematic relations of this organism a word may not be out of place here. since considerable confusion has arisen in the literature regarding the specific name of the organism causing the brown rot of stone fruits (27, 53, 37). Woronin (56) has made an important contribution designed to establish the systematic position of the two species Sclerotinia cinerea and S. fructigena. It has generally been held that S. fructigena causes the brown rot of stone fruits in this country, while in Europe this fungus is found only on pome fruits; but Matheny (37) has recently given good evidence tending to show that it is S. cinerea which causes the brown rot of stone fruits both in this country and in Europe.

#### EXPERIMENTAL STUDIES

#### INFECTION

Some investigators, as, for instance, Zschokke (57), have held that Sclerotinia cinerea is unable to penetrate sound fruit, while Smith (45), among others, has held that the fungus rapidly penetrates and infects sound and unwounded fruit (peaches). Casual observation in the field would seem to justify the former view, for those fruits in contact with other fruits or twigs, and therefore liable to puncture or abrasion, are the ones that are usually found infected; indeed, field observations and laboratory experiments point to the conclusion that infection takes place much more readily, especially with immature fruits, when the cuticle is broken. One would, therefore, naturally raise the question as to whether or not infection can take place when the cuticle is unbroken, and if so under what conditions and in what stages of the development of the fruit. During the summer of 1913

the writer performed a number of experiments which throw more light on the question of the infection of the host.

Methods and Results.-The methods employed were as follows: Plum twigs bearing leaves and fruit were broken off and brought into the laboratory, washed with a mercuric chloride solution (1-1000) and in sterile water. They were then suspended in sterile moist chambers prepared by placing moistened absorbent cotton in the bottom of wide-mouthed one-liter Erlenmeyer flasks that had previously been plugged and sterilized. Twigs having one or more green leaves were used in every case. for in this way green plums hang on the twigs and remain alive This method was especially applicable here, for for some time. it enabled one to maintain absolutely sterile conditions in a moist atmosphere and at the same time keep the host living and in a normal condition. The results of these infection experiments are given in table I.

Discussion of Results.—From these results it is evident that plums were infected as early as June 27, at which time they were immature, in fact not more than half-grown. Infection did not take place when a spore suspension was placed on very green and immature plums unless the epidermis was broken or punc-There were, however, some instances where plums remained healthy in the flask for two or three weeks and became infected only after the lapse of time had brought about an artificial maturity. On the other hand, plums that were approaching maturity, though not mature, as well as mature fruits, may be infected by applying a spore suspension to the natural surface, i. e., a surface which has not been punctured or injured in any way. In this connection it should be mentioned that infection was much more readily accomplished when two plums were hanging so as to be in contact with each other than when they were not touching. This, no doubt, was due to the fact that a drop of water containing spores may be held between the plums long enough for spore germination and infection to take place. These results also indicate that infection takes place readily without puncturing when a portion of the mycelial felt is laid on the surface of either green or ripe fruit.

It should be noted here that one can sometimes find plums in the field only half-grown which are affected with the brown-rot

TABLE I RESULTS OF INFECTION EXPERIMENTS WITH SCLEROTINIA CINEREA

Date	Fruit	Inoculating material	Treatment of surface	Method of inoculation	Results	
June 27	Green plums	Spore suspension	Cuticle killed by steam	Surface application		
June 27	Green plums	Spores	Skin punctured with needle	Needle puncture	+	
July 2	Green plums	Spores	Skin punctured with needle	Needle puncture	++	
July 8	Green plums	Spores	Skin punctured with needle	Needle puncture	++	
July 8	Green plums	Spore suspension	Untreated	Surface application	-	
July 8	Sour cherries	Spores	Skin punctured with needle	Needle puncture	+	
July 23	Green plums	Spores	Skin punctured with needle	Needle puncture	++	
July 23	Green plums	Spore suspension	Untreated	Surface application	-	
July 23	Green plums	Spore suspension	Skin cut	Surface application	++	
July 23	Ripe plums	Mycelium	Untreated	Surface application	++	
July 23	Green plums	Mycelium	Untreated	Surface application	++	
July 30	Green plums	Mycelium	Untreated	Surface application	++	
Aug. 5	Green plums	Spore suspension	Untreated	Surface application	+	
Aug. 13	Nearly ripe plums	Spore suspension	Untreated	Surface application	++	
Aug. 13	ug. 13 Ripe Spore suspension		Untreated Surface application		++	

<sup>\*++</sup> indicates that practically every inoculated fruit became infected. +indicates that only a portion of the inoculated fruits became infected. - indicates that none of the inoculated fruits became infected.

fungus, but so far as the writer's observation indicates, infection in these cases takes place through the twig, or, in some cases, through another plum with which it is in contact and which in turn is infected through the twig. Nevertheless, field observations also verify the laboratory work in that plums (especially may be infected in the field without being in contact with other fruits and without having any visible punctures or wounds in the skin. All these experiments and observations point to the conclusion that penetration of the cuticle is a very important factor in the infection of fruits, especially immature fruits; that infection of very green fruits without punctures is rare; and, on the other hand, that maturing fruits without punctures may be readily infected both by spores and by a mycelial felt in the field and in the laboratory.

#### PENETRATION

The nature of penetration and the course of the hyphæ of parasitic fungi in piercing host tissue is an interesting and important question in connection with a study of the nature of parasitic action. In the case of the brown-rot fungus growing on the plum it is of importance to know whether or not the hyphæ merely follow the middle lamellæ or whether they enter the cells wherever they come in contact with them. Previous investigators differ very widely in their opinions as to the nature and course of the penetration of the fungus in question, a condition which is perhaps partly explained by the fact that different hosts were employed in the various investigations. Furthermore, it appears that the methods employed in some of the researches were not of such a character as to readily yield complete information concerning all the facts in the case.

In my own work a study of the penetration of the host tissue by the fungus was made by examining a number of sections of infected tissue in which the disease had reached various stages of development, and comparing them with sections of healthy tissue from the same fruit. For this purpose a special method was employed.

Methods and Results.—Small pieces of fruit composed of diseased and sound tissue were cut from plums inoculated with

a pure culture of the fungus. These segments were immersed in 70 per cent alcohol just long enough to partially kill the fungous filaments and the host cells, yet not long enough to discolor the sound tissue or to modify or change the color of the diseased tissue in any way. From this material razor sections. containing both diseased and healthy tissue, were made, stained for a short time in eosin, and then partially destained with alcohol. If the pieces of plum had not remained in the alcohol for a sufficient length of time, the razor sections were immersed in 70 or 95 per cent alcohol before staining. By employing this method it is possible to stain the fungous filaments deeply. while the host tissue remains unaffected. Indeed, this method permits of a rather sharp color differentiation between the healthy and the diseased tissue, the latter being blackened by the disease. This method, though quite applicable for the purpose at hand, was primarily developed for another purpose, which will be discussed below.

Since every fungous filament is very sharply differentiated, one may readily study the course of the hyphæ with reference to the host cells. By staining, sectioning, and examining diseased material taken from the margin of the infected area, one finds the fungous hyphæ penetrating the cells at any point of contact; indeed, after examining a number of specimens by the method reported above, the writer finds no indications that the fungous hyphæ follow the middle lamellæ, as has been reported by other investigators (57, 6) for pears and other fruits. The above method also enables one to contrast the cell walls of infected and penetrated cells with those of normal tissue. It is entirely possible that the fungous filaments, on coming in contact with a cell wall, secrete just enough enzyme to dissolve their way through the cell walls, leaving the walls of the host cells surrounding the hyphæ entirely normal, i. e., without swelling or disorganization.

Another and somewhat different experiment was performed to get additional evidence on this point. From sound plums which had previously been rendered sterile by washing in bichloride of mercury solution (1–1000) and sterile distilled water, free-hand sections were cut with a razor sterilized in 50 per cent alcohol. The sections were arranged in hanging drop cultures

and each inoculated with a drop of a very dilute spore suspension containing two or three spores per drop. The progress of the fungus and the condition of the host cells were noted from day to day but no visible disintegration of the cell walls could be observed, nor did the fungus show any particular affinity for the middle lamellæ.

Conclusions.—We would conclude, therefore, as a result of direct observation on the host tissue, that the fungus penetrates the host very readily and rapidly, that it does not necessarily follow the middle lamellæ in the plum and the peach, and that there is no visible general disintegrating action on the middle lamellæ or on the cell walls of the living host.

#### ACTION OF THE FUNGUS ON THE LIVING HOST CELLS

A significant fact in the metabolism of the brown-rot fungus is that it induces such an exceedingly rapid decay in the infected This rapid decay might be connected both with a rapid growth of the fungus and with a pronounced power which the organism possesses of breaking down and changing the constituents of the host. Moreover, several representatives of the genus Sclerotinia have been reported to have the power of secreting an enzyme or some other substance which kills the host cells in advance of penetration. Were this the case, it would be expected that rapid decay would accompany the action of the parasite. Is this view applicable to the action of Sclerotinia cinerea? The investigators who have made a study of this organism differ very widely in their views regarding the effect which it has on the host tissues, and it seemed desirable, therefore, to determine the relation of hyphal penetration to the death of the cells.

Methods and Results.—In order to fix the material for this study, it was found satisfactory to proceed as follows: Small pieces of the host tissue were taken from the margin of the diseased area and placed in 95 per cent alcohol for a short time. Free-hand sections were made of this material so as to include both diseased and healthy cells, and the sections stained for a short time in eosin and subsequently decolorized in part with alcohol, if necessary to give the desired contrast. By this

method the fungus may be distinctly differentiated from the host tissue, the killing and staining agents having little or no effect on the host cells. There is a more or less sharply differentiated line of demarcation between the injured and the sound cells, as indicated by the darker color of the former. The effect of the fungus is readily discerned by the blackening of the host tissue, this being especially noticeable in green plums. discolored and poisoned cells are not at first plasmolyzed, and it is to be noted here that discoloration rather than plasmolysis should be taken as the index of the toxic action of this fungus on its host. It should perhaps be mentioned here, too, that the blackened cells shade off somewhat gradually into the hyaline healthy ones, and that, therefore, there is not always a sharp line of demarcation between the diseased and the healthy cells. However, in spite of these difficulties, I was convinced, after having examined a large number of sections of diseased and healthy tissue, that there is no positive evidence that the host cells are discolored, and therefore injured and poisoned, in advance of actual penetration by the fungus.

The indirect method employed to determine the same point consisted in applying to sound fruits an extract from decayed plums. Fruits were disinfected with mercuric chloride solution, washed in sterile distilled water, and inoculated with Sclerotinia cinerea. When the plums had become thoroughly decayed the juice was extracted and filtered under sterile conditions through a Chamberlain filter. The juice thus obtained was incubated for one week at a temperature of 22-25° C., and also tested on nutrient agar plates, and found to be sterile by both methods. From sound plums, which had been disinfected in the usual manner, a cone-shaped plug was cut out and the resulting cavity filled with this sterile extract,—the controls being prepared in a similar manner, using sterile water instead of the plum extract. The results were negative, that is, the controls were not unlike those treated with the extract from decayed plums.

The same experiment was repeated in a modified form by using thin razor sections of both green and ripe plums, the sections being made under sterile conditions as before, and observed in a hanging drop of sterile juice from decayed plums.

By means of this method one could readily observe any changes that might take place in the cells and make accurate comparisons with controls. Frequent observations were made, and throughout this experiment, which continued for several days, one could not distinguish between the appearance of those sections in a drop of sterile water and those in the sterile extract from decayed plums. It is possible and perhaps probable that this fluid, being merely the juice of the fruit, was too dilute to be effective, but the experiment was made because of the possibility of positive evidence.

Discussion of Results.—The initial stage in the injury caused by this fungus is shown by discoloration only and not by plasmolysis, and therefore one cannot draw conclusions with absolute certainty as to the poisoning effect of the extract on the cells of a cut surface, for the latter turn brown as soon as exposed to the air, just as when infected with the organism. It was comparatively easy, however, to observe that the extract had no effect on the cell walls, for no difference could be observed between the cell walls of the tissue thus treated and those of the control specimens. Even where the sections were left in the extract for several days neither swelling nor disorganization of the cell walls or middle lamellæ was noted. When sections of plum tissue were inoculated with one or more spores of the brown-rot fungus no cell-wall disintegration resulting from the growth of the fungus could be observed. A comparative study of sections of tissue, respectively exposed and not exposed to the action of the extract from decayed fruit, showed that no difference could be detected between the two, and that, therefore, no enzyme with a perceptible cytolytic action exists under these conditions. It has been held by some, notably by Behrens (6), that the injury to the host cell is largely physical in that the fungus penetrates at such a prodigious rate that the fluids of the host cell are allowed to escape with loss of turgor to the latter; furthermore, that the osmotic equilibrium is soon destroyed, with plasmolysis and death ensuing. It is very probable that part of the rapid injury to the host can be explained on purely physical grounds, but this may not be the only factor involved, although we do not now know what chemical activity of the fungous cells may be concerned in the rapid killing of the host tissue.

### ACTION OF THE FUNGUS ON CELLULOSE

A number of investigators have regarded cellulose dissolution as a very important factor in the parasitism of many fungi; indeed, some of the earlier workers seemed to consider this the prime factor involved. While it is a well known fact that there are many fungi, especially saprophytes, which hydrolyze, or dissolve, certain celluloses, research extending over a wide field has revealed the nature of parasitism to be a very complex one in which other factors are as important as the dissolution of cellulose and the cell wall.

It has been the writer's purpose to study from two different points of view the action of the brown-rot organism on celluloses, (1) by observing the action of the fungus on pure cellulose isolated from the host tissue, and (2) by studying microscopically its action on the host cell walls themselves. In the former study cellulose agar was used, the cellulose being isolated from plums by the methods discussed below.

Methods and Results.—In the above mentioned study of the action of the fungus on pure cellulose, a variety of reagents, media, and methods for the preparation of cellulose were employed, a brief account of which follows. Schweizer's reagent was prepared by adding a slight excess (40 grams to the liter) of copper carbonate to dilute ammonium hydroxide solution composed of three parts of water to ten parts of ammonium hydroxide (sp. gr. 0.90). The copper solution was then shaken vigorously, allowed to stand over night, and the supernatant solution siphoned off. This is the procedure employed by McBeth and Scales (38).

Paper cellulose from filter paper was prepared according to the method given by McBeth and Scales (38) by dissolving 15 grams of sheet filter paper in Schweizer's reagent, diluting about ten times with water, and precipitating the cellulose with a solution of one part of hydrochloric acid to five parts of water. This mixture was then further diluted to 15 or 20 liters, the supernatant liquid siphoned off, and the residue washed repeatedly with water until the precipitated cellulose was free from both copper and chlorine. After standing quietly for several days the clear liquid was siphoned off and the precipitate used for the preparation of cellulose agar.

Cellulose agar was made by adding about one per cent (estimated by the weight of the paper before treating with Schweizer's reagent) of precipitated paper cellulose, prepared as stated above, to a mineral nutrient solution, the complete medium having the following composition:

Cellulose suspension	500 cc.
Agar	10 grams.
Monopotassium phosphate, 1 gram	
Magnesium sulphate, 1 gram	
Sodium chloride, 1 gram	
Ammonium sulphate, 1 gram	[
Calcium carbonate, 2 grams	
Tap water, 1000 cc.	1

The insoluble precipitate appearing in the mineral nutrient solution was filtered off before the cellulose suspension and agar were added. Good results were also obtained by using 0.5 gram of calcium nitrate instead of 2 grams of calcium carbonate, in which case filtering is unnecessary. The mineral nutrient solution having the composition tabulated above will be referred to as nutrient "A."

Another nutrient solution very low in organic matter was also employed in the cellulose agar, but with rather unsatisfactory results. This solution, which will be referred to as nutrient "B," is that employed by Reed (42), and is made up as follows, the only organic material present being the small amount of sodium citrate:

Ammonium nitrate	10	grams
Dipotassium phosphate	5	grams
Magnesium sulphate	1	gram
Sodium citrate	1	gram
Tap water		cc.

In making the cellulose agar this nutrient solution was used in exactly the same way as nutrient "A."

Since previous investigators have held that the celluloses from various sources differ in their resistance to hydrolyzing enzymes, an attempt was made in this investigation to prepare a cellulose from a natural host—plums—of the parasite. In order to secure a cellulose that is modified as little as possible in the process

of isolation three different methods were employed in preparing cellulose from plums, the resulting products being designated, for convenience in reference, respectively as soda cellulose, washed cellulose, and potassium chlorate cellulose.

In the preparation of soda cellulose ripe plums were squeezed through cheese cloth and the pulp was washed thoroughly with water. The pulp was then treated with an 8 per cent solution of sodium hydroxide and heated in the autoclave at ten pounds pressure. After thoroughly washing the pulp with water the heating with alkali was repeated and the product given final washings until free from alkali.

The second method of isolating cellulose—washed cellulose—consisted in washing the fruit pulp with water until free from substances soluble in cold water. Water was then added and the mixture heated in the autoclave at 15 pounds pressure, and washed. The operation was repeated as long as any water-soluble substances could be detected. This method, of course, gives an impure cellulose, yet the product is one that is free from water-soluble substances.

The third method consisted in oxidizing, dissolving, and washing out the plum pulp until a pure cellulose—potassium chlorate cellulose—was obtained. Pulp, secured from ripe plums in the manner stated above, was washed with cold water until the wash water was free from solutes, and then treated with a cold solution composed of 30 grams of potassium chlorate dissolved in 520 cc. of cold nitric acid (sp. gr. 1.1). This mixture was kept in the ice box for about three weeks, at the end of which time the pulp was entirely white. This method is said to yield a product that differs only very slightly from the original cellulose.

The product obtained by these various methods was not allowed to dry, for it is possible that drying changes the nature of cellulose so that it is more resistant to the action of cytolytic enzymes. A part of the cellulose obtained by each of the preceding methods was treated with Schweizer's reagent and precipitated with hydrochloric acid and washed as stated above under the preparation of filter-paper cellulose. These three cellulose preparations thus treated with Schweizer's reagent, as

<sup>&</sup>lt;sup>1</sup> Fowler, G. J. Bacterial and enzymatic chemistry. 159. 1911.

well as the three corresponding untreated portions, were used in the preparation of cellulose agars, according to the method given above. The media were placed in test tubes of very small (8 mm.) diameter, and sterilized. The tubes of melted agar were then cooled rapidly in cold water in order to bring about the hardening of the agar before the cellulose had had time to settle to the bottom of the tubes.

Tubes of the various cellulose agars were inoculated with Sclerotinia cinerea and others with a species of Penicillium. which will be designated as P. expansum<sup>1</sup>, isolated from decaying peaches and apples. Since these two fungi, viz., Sclerotinia cinerea and Penicillium expansum, act very differently toward the host, a word contrasting their action may not be out of place here. As a result of inoculating apples, peaches, or pears with a pure culture of Sclerotinia the host tissues are promptly killed, while the fruits remain practically as firm after complete decay as before inoculation. On the other hand, the fruits inoculated with the Penicillium become very soft and watery, developing a pustule or sunken area where the infection took place. One may assume, therefore, that the Sclerotinia does not materially affect the celluloses and pectic substances that make for the firmness of the fruit, while, on the other hand, Penicillium does affect these substances, causing the fruit to lose its firm consistency. Since these two fungi show such entirely different and opposing characteristics as regards their effect on the same host, it is interesting to compare their action in pure cultures on cellulose and pectin-like substances. Such a comparative study was made, the results of which are given in table II.

Discussion of Results.—The results given in table II indicate that both Sclerotinia cinerea and Penicillium expansum exhibited in general a very slight hydrolytic action when grown on cellulose isolated from the plum, there being very slight action with both fungi on the soda cellulose and also on the potassium chlorate cellulose and no action on the washed plum cellulose. On the other hand, both fungi very readily dissolve filter-paper

<sup>&</sup>lt;sup>1</sup> A culture of this organism was sent to Dr. Chas. Thom, who very kindly examined it and gave as his opinion that it was *P. expansum*, or perhaps a strain of that species. The organism in question, when grown on the media employed by Thom, showed characters very similar to those of *P. expansum*, as given by Thom (48).

TABLE II
ACTION OF SCLEROTINIA CINEREA AND PENICILLIUM EXPANSUM ON CELLULOSE

	Nutrient solution added	Sclerotinia cinerea		Penicillium expansum	
Type of cellulose used		Growth	Cellulose hydrolysis	Growth	Cellulose hydrolysis
Soda cellulose	A	++†	-†	++	+
Soda cellulose	В	++	-		
Potassium chlorate cellulose	A	++	+	++	+
Potassium chlorate cellulose	В	++	+		
Washed ligno-cellulose*	A	+	_		
Washed ligno-cellulose*	В	_	_		
Washed cellulose	A	+	-	+	_
Soda cellulose (Schweizer's)	В	+	-	+	_
Soda cellulose (Schweizer's)	A	++	+		
Washed cellulose (Schweizer's)	A	+	_		
Soda cellulose	Peach juice	+++	_	+++	-
Filter paper strips	Peach juice	+++		+++	-
Filter paper strips	A	++	_	++	-
Filter paper strips	В	+	-	++	-
Filter paper strips	0.5% glucose solution	+++	-	+++	-
Filter-paper cellulose	A	++	+++	++	+++

\*Ligno-cellulose is the name here given to cellulose from the vascular tissues of the plum, i. e., that part of the pulp which did not go through the cheese cloth.

†Growth and cellulose hydrolysis are indicated by +, the relative intensities of growth and degrees of hydrolysis being indicated by one or more + marks. Absence of growth and absence of hydrolysis are indicated by —.

cellulose, and, strange to say, Sclerotinia is just as active in this respect as Penicillium. In many cases the growth was as good on the plum cellulose as on the filter-paper cellulose, yet the hydrolytic action of the fungi was very much weaker on the former medium. No cellulose hydrolysis occurred where peach juice or some soluble carbohydrate, such as glucose, was added. It seemed probable at first that a very small amount of glucose, or peach juice, or sodium citrate would give the fungus a vigorous start and thus accelerate its cyto-hydrolytic activity, but the quantities of these substances employed was sufficient to exert a protective influence, there being a vigorous growth but

no apparent cellulose hydrolysis:

The fact that these fungi do not dissolve cellulose, derived either from the host or from paper, when other organic nutrients are supplied, verifies the writer's observation that Sclerotinia cinerea does not disintegrate the cell walls of the host tissues. Furthermore, the fact that the fungus dissolves paper cellulose very readily when it is the only carbohydrate supplied, leads one to conclude that the action of the fungus on paper cellulose in a nutrient solution low in carbohydrates is not necessarily a good criterion for judging the behavior of the fungus in the host tissue. In the host tissue there may be a form of cellulose different from that of paper, and it is furthermore very evident that there is present in the fruit an abundance of organic material evidently operating in a protective manner. The fungus fails to produce cytolytic enzymes when grown on plum or paper cellulose to which peach juice or even a very little sugar has been added, but acts vigorously on paper cellulose to which no organic nutrient has been added. It is rather peculiar that both fungi act much more readily on paper cellulose than on cellulose isolated from the fruits which are natural hosts for these organisms.

Sclerotinia cinerea grows very slowly when first transferred to a nutrient medium poor in soluble carbohydrates, very few spores and no aërial mycelium being produced. At the expiration of a week or more one may observe that the fungous mycelium has penetrated the surface layer of the agar, and at the expiration of two to three weeks, in case the fungus is growing on paper-cellulose agar, a clear translucent ring may be observed

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in the agar just below the fungous filaments, thus indicating that the cellulose is being hydrolyzed. With increasing age of the fungus, this clear and almost transparent area gradually enlarges downward, although the fungus shows little or no corresponding penetration. At the expiration of three weeks or a month, there is a very distinct, clear, and nearly transparent zone in the medium below the region occupied by the fungous mycelium. Since one could see very distinctly how far the fungous filaments had penetrated into the substrate, it was very evident that the cyto-hydrolytic enzyme had diffused beyond the limits of the mycelium.

The method employed in this investigation for the demonstration of cellulase was the same as that used by Kellerman in his recent work (31) and was utilized to demonstrate the fact that the cyto-hydrolytic enzyme secreted by this fungus penetrates the substrate considerably beyond the limits of the filaments themselves. Tubes containing cellulose agar, in which the fungus had been growing for four weeks, were disinfected externally by washing with a bichloride of mercury solution, and cut off at a point about 12 mm. below the clear portion of the medium. The cotton plug was then flamed and pushed into the tube with a glass rod until the agar was partially shoved out of the cut end of the tube. The clear portion of the agar was then cut into disks about 12 mm. in thickness, which were laid on plates poured with nutrient cellulose agar, great care, of course, being exercised throughout the operation to maintain aseptic conditions. The plates so prepared were then placed in an incubator at 25°C. where they remained for two weeks, at the expiration of which time the cellulose was very distinctly hydrolyzed in a ring about the sterile slices of agar. Microscopic examination confirmed the macroscopic observation that these agar disks were free from any infection.

As might be expected, the activity of the secretion of the enzyme cellulase is influenced by temperature, a fact which is well illustrated by the following experiment: Tubes containing cellulose agar inoculated with the brown-rot fungus were kept at temperatures of 10–12, 16–20, and 24–26°C. respectively, and at the end of twelve days the following results were noted: In the cultures maintained at 10–12°C. no apparent growth or

hydrolysis had taken place; those kept at 16–20°C. showed a good growth but no visible cellulose hydrolysis; and in those maintained at 24–26°C. there was about the same extent of growth as in the preceding series but accompanied by a very evident cellulose hydrolysis, a distinctly clear zone of dissolved cellulose surrounding the region occupied by the fungous mycelium. It is therefore evident that even with approximately the same amount of growth cellulose hydrolysis is much more rapid at the higher temperature.

An effort was made to determine whether or not it is possible to "train up" more active cyto-hydrolytic strains of the Sclerotinia and Penicillium in question. On the one hand, these fungi were grown for several successive generations on peach-juice agar—a medium in which the organisms show no cytolytic activity. On the other hand, these fungi were cultivated for several successive generations on paper-cellulose agar-a medium which is low in soluble carbohydrates, and one in which the fungi exhibit considerable cytolytic activity. Tubes of papercellulose agar were then inoculated with the fungi grown in these two ways and careful observations were made to detect any differences in cyto-hydrolytic activity. No differences developed, however, from which it would appear that the source of cultures of Sclerotinia or of Penicillium does not materially affect the cellulose-dissolving capacity of these organisms, i. e., each fungus shows the same cellulose-hydrolyzing power whether the organism was cytolytically active during the immediately preceding generations or not.

#### EFFECT OF THE FUNGUS ON PECTIC SUBSTANCES

The power of organisms to change pectic substances has been considered an important factor in the disintegration and softening of host tissue by certain plant parasites. Before entering into a discussion of the experimental phases of this subject, it will perhaps be well to give some idea of the present status of this question, as well as a very brief resume of the extensive literature which has accumulated about it.

Fremey (23, 24), in 1840, was the first to report an enzyme acting on pectic substances. This enzyme, which he isolated and called pectase, induced the coagulation of pectin, Fremey attrib-

uting this action of the enzyme to the presence of calcium salts. It is of interest to note that pectase was one of the first plant enzymes to be described. Bertrand and Mallèvre (7, 8) concluded that pectose and pectase are almost universally present in green plants, being especially abundant in the leaves. These authors showed that acidity is an important factor in the inhibition of coagulation of pectic bodies by pectase, and also that either barium, calcium, or strontium is necessary for the action of pectase.

Mangin (35, 36), by microscopic tests, has thrown much light on the nature of the middle lamella and holds that pectose is very pronounced in the cell walls of young tissue. In the older cell walls, on the other hand, this author believes that calcium pectate predominates in the middle lamella, considering that the latter is largely if not entirely composed of this substance and that it frequently collects on the surface of the cell walls adjoining intercellular spaces. Bourquelot (11), and Bourquelot and Hérissey (12) secured a thermo-labile enzyme from barley malt extract which acted upon a solution of pectin (taken from the gentian root), changing the latter in such a way that it was no longer coagulated by pectase. The action of this enzyme, which they called pectinase, was thought by them to be that of converting the pectin into reducing sugar. They also designated as pectinase an enzyme which dissolves the pectic coagulum (the latter has been supposed to be calcium pectate). A good resumé of the status of the chemistry of pectic substances is given by Bigelow and others (10).

A number of investigators have reported upon the action of bacteria on plant cells, including the effects of the organisms on the middle lamella. Winogradsky (55), Behrens (5), and others attributed the changes taking place in the flax plant during retting to the dissolving action which the bacteria exert on the middle lamella. It will be unnecessary to review here any more of the earlier work which has been done along this line, since it has been so thoroughly discussed in the comprehensive publications by Jones (29), and Jones, Harding, and Morse (30) on the soft rot of vegetables. These authors studied the effect of the soft-rot bacillus (Bacillus carotovorus) on the host and find that the organism is identical with what has been

designated as B. oleraceæ Harrison, and B. omnivorous Van Hall, and that it may possibly be identical also with Potter's B. destructans. By many tests Jones has shown that this organism secretes an enzyme which causes the disintegration of the host cells by dissolving the middle lamella, which, according to the majority of investigators, is composed of salts of pectic acid. This author has further isolated from pure cultures of the organism an extra-cellular enzyme, which he designated pectinase, that destroys the middle lamella of the cells just as does the growing organism. Jones, therefore, considers this enzyme responsible for the disintegrating action of the bacillus.

In my own work I shall adopt the nomenclature used by Jones (29, 30) and Euler (21), namely, employing pectinase as the term to designate the enzyme inducing coagulation of a pectin solution and also the hydrolysis of calcium pectate, or pectinate.

Methods.—In order to determine the effect of the fungus on the middle lamella I have used two methods, (1) a microscopic study of the effect of the fungus on the host cells, and (2) a study of the effect of the organism on the substances (isolated from the host) which are commonly reported to be constituents of the middle lamella. The first method has been discussed above and may be dismissed here by stating that it yielded no positive evidence that the fungus dissolves the middle lamella. By the second method the problem was studied by isolating pectin from the host and studying the effect of the fungus on it and also on its salts, as, for instance, calcium pectinate.

Pectin was isolated from plums by the following method: Thoroughly ripe fruits were steamed—no water being added, the juice filtered off and treated with Almen's reagent¹ (to precipitate the protein) and with a very dilute solution of oxalic acid (to precipitate the calcium). It was found that under these conditions neither a calcium nor a protein precipitate was thrown down either by Almen's reagent or the oxalic acid, and this procedure, therefore, was deemed unnecessary and was abandoned. The plum juice was carefully filtered through a Buchner filter

<sup>&</sup>lt;sup>1</sup>Abderhalden, E. Handbuch d. biochem. Arbeitsmethoden 2: 391-92. 1910. Almen's tannic acid solution is made by treating 4 grams of tannic acid with 8 cc. of a 25 per cent solution of acetic acid, and making up to 190 cc. with 40 or 50 per cent alcohol.

and the filtrate treated with 95 per cent alcohol until a flocculent coagulum of pectin was produced. This pectin was separated by means of a Buchner filter, redissolved in water, reprecipitated with alcohol, again separated by means of a Buchner funnel, and finally dried at a temperature slightly higher than room temperature,—the reprecipitation being for the purpose of purification. It should be noted here that the plums were sufficiently acid to make the addition of hydrochloric acid to the alcohol unnecessary.

Experiments with pectin and pectinase.—From the pectin isolated by the above method a saturated aqueous solution was prepared—some of the mineral nutrient solution1 minus calcium being added, and the resulting solution rendered sterile by fractional sterilization. Test-tubes of this pectin solution were inoculated with Sclerotinia cinerea and Penicillium expansum with the result that both organisms produced a rather vigorous growth of mycelium and a few spores. At the expiration of one week the inoculated tubes showed a slight clear area just below the fungous felt due to the coagulation and settling out of the pectin in that part of the solution. The coagulation was at this time somewhat more pronounced in the Penicillium cultures than in those of Sclerotinia, yet very noticeable in both cases, beginning directly below the fungous felt and progressing toward the bottom of the tube. After two weeks the greater part of the pectin solution was coagulated, the flocculent coagulum, or precipitate, being very different from the precipitate produced in a pectin solution by a calcium salt. It should be emphasized here that every precaution was taken to maintain a calcium-free solution, and when it is considered that the addition of calcium develops a reaction very different from that produced by the enzyme, and, furthermore, that the check gave no coagulation whatever, not even when allowed to stand a month or more, the conclusion would seem to be warranted that calcium is not necessary for the production of a gel by pectinase. Both Sclerotinia and Penicillium, therefore, produced a coagulum in an aqueous solution of pectin, while no such results were obtained in the controls, thus justifying the conclusion

<sup>&</sup>lt;sup>1</sup>Nutrient solution employed was the same as mineral nutrient solution A used in preparing cellulose agar, but without the calcium.

that these two fungi are capable of producing pectinase. The cultures were kept at a temperature of 18-20°C.

Experiments with calcium pectinate.—Calcium pectinate was prepared by treating a water solution of pectin with freshlymade limewater (care being exercised to avoid an excess of lime). the product thus obtained being filtered off and thoroughly washed until it was no longer alkaline. The calcium pectinate thus prepared was used in making a pectinate agar in a manner similar to that employed in the preparation of cellulose agar. the same mineral nutrient solution (nutrient A) being used and the whole rendered sterile by fractional sterilization. After the last heating, care was taken to distribute the pectinate. which quickly settles to the bottom of the tubes, uniformly throughout the agar by stirring the medium with a sterile glass These tubes were then inoculated with Sclerotinia and with Penicillium, the object being to compare the action toward pectic substances of two fungi that have entirely different effects on the host cells, the former producing no softening effects, while the latter causes a very rapid softening and disorganization of the host tissue.

The inoculated tubes of pectinate agar prepared by the above method were kept at a temperature of 22–24°C. Contrary to expectations, there was very little growth when no soluble carbohydrate was supplied, and, furthermore, no dissolving action on the calcium pectinate. On the other hand, when 0.5 per cent glucose was added, both fungi produced a vigorous growth, but neither one gave any indication of pectinate hydrolysis, or dissolution. Here again, as in the cellulose hydrolysis, the two fungi, *Sclerotinia* and *Penicillium*, behave alike. This is not in accordance with the observed behavior of these two organisms toward the host tissue.

#### ACID RELATIONS OF THE FUNGUS

Some investigators have held that the content of tannin (47) and of malic and other acids of the host determines whether or not the fungus can grow in the tissues and rot the fruit. In accordance with this view a fungus may not so readily attack green as ripe fruit, the former being supposed to exhibit a higher

content of these restraining agents. The question of the acid relation of the host tissue is one of fundamental significance and one that is worthy of considerable investigation; it is important to know to what extent acidity may be a limiting factor in parasitism.

A case in which a certain acid content is favorable for the fungus is developed by Falck (22). He finds the acidity of the substrate to be a conditioning factor for the growth of several species of Merulius. In this connection the author observes that Coniophora, in particular, acts to pave the way for Merulius in that the former organism renders the nutrient substrate decidedly acid, and thereby provides favorable conditions for the germination of the spores and the subsequent growth of mycelium and fruit bodies of Merulius. In connection with the investigation of the plum disease here discussed it would be well to know if the acidity of the fruit changes during the progress of its growth, and if so in what direction. It is also essential to know whether or not a change in the acidity of the host can account for the fact that ripe fruit is more susceptible to the disease than green fruit. Some experiments were planned, therefore, to determine to what extent the acidity of the host influences the attack of the parasite, and also to investigate what effects, if any, the fungus has with respect to the acid content of the host.

In order to determine the changes in acidity which take place during the growth of the fruit (plums), several analyses for acidity were made at intervals during the summer. The plums for all of the analyses were taken from the same tree, a known weight of pulp being ground up in a mortar and squeezed through muslin. The acidity was reckoned in the number of cc. of N/10 NaOH required to neutralize one gram of plum pulp. The results were as follows:

- June 28, 1 gram plum pulp required 0.66 cc. N/10 NaOH for neutralization,
- Aug. 2, 1 gram plum pulp required 2.12 cc. N/10 NaOH for neutralization,
- Aug. 19, 1 gram plum pulp required 2.46 cc. N/10 NaOH for neutralization,

the fruit being market ripe on August 19. In these tests my results agree with those obtained by Bigelow and Gore (10) for peaches, and with those of Thompson and Whittier (49) for some other fruits. The last mentioned investigators, however, found that the acidity of peaches decreases toward maturity. I have been unable to secure data covering the acidity of plums throughout the season.

The above results show that the acid content of plums increases rather than diminishes toward the maturity of the fruit. The results of the experiments and field observations show that mature and ripe fruit is much more susceptible than the green and immature fruit. The above facts, showing that as the fruit approaches maturity the acidity increases while the susceptibility to the disease also increases, indicate that there is no close relationship between the low acid content of the host and susceptibility to the brown-rot fungus, and that we must look to other factors to explain infection as observed in the field. As pointed out, my experiments indicate that penetration is a

TABLE III
RELATION OF THE GROWTH OF SCLEROTINIA CINEREA TO THE REACTION OF THE MEDIUM

Medium	Acidity	Growth after 8 days	Growth after 16 days	Spore production		
Cherry juice	+2.3*	-t	+†	+		
Cherry juice	+1.5	++	++	++		
Cherry juice	+1.0	+++	++	++		
Cherry juice	+0.15	-	+	+		
Cherry juice	-0.15	0	++	0		
Cherry juice	-0.30	0	++	0		

<sup>\*</sup>Acidity is given in cc. of N/10 NaOH necessary to neutralize 1 cc. of the juice.  $\dagger$ The + sign indicates a fairly good mycelial growth, or spore formation, and the - sign indicates that the growth was just perceptible; 0 indicates no growth, or no spore formation.

very important factor. It is possible that a study of the tannin content<sup>1</sup> might yield some relation of interest.

A preliminary experiment was planned to determine the acidity at which the optimum growth and spore production of the fungus occurs. For this purpose the juice from ripe sour cherries was used. The juice was squeezed out of the cherries (no water being added) and a portion titrated to determine the acidity. Then 50 cc. of this liquid were put into each of a number of Erlenmeyer flasks of 125 cc. capacity; some of the flasks were left untreated, while others received various quantities of N/10 NaOH to bring each to the desired acidity or alkalinity. The flasks were then sterilized and inoculated. The results are given in table III.

It is clear, therefore, that although the fungus eventually grows on a medium as acid as the natural juice of sour cherries, it grows more luxuriantly on a somewhat less acid medium. It is a rather significant fact that on the media near the neutral line the fungus at first shows no perceptible growth, but at the expiration of two weeks has produced nearly as much mycelial growth as on the acid medium. It is also of interest to note that we find spore formation abundant on the very acid media but entirely lacking on the alkaline media. This experiment indicates that the fungus can adjust itself to a slight degree of alkalinity.

#### OXALIC ACID PRODUCTION BY THE FUNGUS

The first important reference to oxalic acid production by fungi is in the publication by de Bary reviewed in a preceding section. He reports that the older hyphæ of the fungus were encrusted with crystals of oxalic acid, and he attributed some of the poisonous action of the parasite to the production of this substance; in fact, he mentions oxalic acid fermentation. Since the appearance of de Bary's paper a limited number of investi-

<sup>&</sup>lt;sup>1</sup>Cook and Bassett and their associates (17) believe that there are enzymes in the host plant which may act upon cell constituents and play the rôle of alexins. They are of the opinion that tannin, as such, is not abundant in fruits, but that it may be formed by the action of oxidizing enzymes upon certain phenols. Injuries produced by parasitic fungi may accelerate the activity of the host in the production of tannin, the latter perhaps being toxic to the growth of parasitic fungi.

gators have reported the presence of oxalic acid resulting from the growth of both fungi and bacteria, but unfortunately much of this work is of little value, because methods of analysis are not given. The detection of this acid by some methods is very unsatisfactory.

A few years after de Bary's work, Wehmer (54) published an extensive series of articles on this subject. He studied a number of fungi (mostly saprophytic) with reference to oxalic acid excretion, and of these he found Aspergillus to be the most active and Penicillium next, and, therefore, he confined his studies to these Some of the factors concerned in the production of oxalic acid or its salts, according to Wehmer, may be summed up here: (1) A large yield of oxalic acid is not produced in the presence of free organic or inorganic acids, not being found in the medium when free acids exceeded 0.2-0.3 per cent, while, on the other hand, it can be formed in the presence of as much as 2-3 per cent of the salts of these acids. (2) The sources of nitrogen are very important, for the amount of the oxalic acid produced varies according to the kind and quantity of nitrogenous compounds supplied. (3) Abundant oxalic acid formation is favored by the addition of some basic phosphate, or at least some compound with which the acid can combine to form a soluble salt. (4) The effect of light or darkness on oxalic acid formation is inappreciable. (5) Temperature is an influencing factor in oxalate production, for the latter is inhibited by a high temperature, the temperature for a maximum oxalate production being, in fact, very near the minimum for the growth of the organism.

Wehmer's analytical method consisted in precipitating out the oxalic acid, or its soluble oxalate, as the calcium salt, which was filtered off, dried to a constant weight, and weighed. Although this method is perhaps as well suited for this purpose as any other reported, it is open to criticism. A detailed discussion, however, will not be given here.

Wehmer holds that oxalic acid is a type of excretion, and that it is in some way connected with respiration, that is, with CO<sub>2</sub> elimination. He considers that the variability in the amount of oxalic acid produced is due to its use in the metabolism of the fungus. Emmerling (20), in his contribution to this subject,

emphasizes the influence of such nitrogenous substances as proteins, amino acids, and amides in the nutrient. He finds that Aspergillus niger when grown in non-amino acids, for example, tartaric, lactic, etc., produces no oxalic acid, whereas an abundant oxalic acid production results on such substances as peptone or aspartic acid.

Smith (46) and Peltier (41) both conducted experiments to determine whether or not oxalic acid is present in media in which Botrytis has been growing. Peltier reported negative results, but Smith found oxalic acid and thinks that the poisoning effect of the fungus is perhaps due to the presence of this acid. Unfortunately, neither of these authors gives his methods of analysis, and, with the exception of one incident in Smith's publication, the quantity of oxalic acid found is not reported. Peltier and others have been able to produce an injury with oxalic acid similar to that produced by certain parasitic fungi, such as Botrytis, yet this is not conclusive evidence that oxalic acid is the toxic substance secreted by the organism.

The articles mentioned above constitute the chief publications that have to deal with the production of oxalic acid by fungi. The publications on the production of oxalic acid by bacteria and other plants will not be reviewed here. Whether oxalic acid production is a phenomenon peculiar to certain genera or to certain species of the fungi, whether it is purely the result of external conditions, or whether it results primarily from certain constituents of the medium, has not been clearly demon-A series of experiments was planned in the hope of throwing some light on its production in the fungus here studied.

The method of analysis employed was a modification of Wehmer's method of precipitating the oxalate with calcium chloride and determining the amount of oxalate thus precipitated. This method, however, is not well adapted to the purpose at hand, especially when quantitative methods are used, and fruit juice is employed for the medium on which to grow the fungus. An attempt is being made to develop a method that will be better suited to our purpose.

Culture media were prepared from peaches and plums by filtering the juices of these fruits through a Hill pressure filter under sterile conditions. The product thus obtained was placed in flasks and incubated for a week and found to be sterile, after which the flasks were inoculated with *Sclerotinia cinerea*. At the expiration of thirty-seven days these cultures were analyzed and were found to contain the following amounts of oxalic acid per 50 cc. of the respective juices:

Plum juice.	 					0.0019	grams	of	oxalic	acid,
Peach juice	 	 				0.0077	grams	of	oxalic	acid,
Peach juice	 	 				0.0094	grams	of	oxalic	acid,
Control	 	 				N	o trace	e of	oxalic	acid.

Plum and peach juices that had been sterilized by heat, thereby precipitating some of the contained proteinaceous material, were also used as culture media, and here, too, every culture containing the fungus gave a positive test for oxalic acid.

For investigating the production of oxalic acid by the fungus in the unaltered fruit, lots of 500 grams each of peaches were disinfected with bichloride of mercury solution, inoculated respectively with Sclerotinia, Penicillium, and Aspergillus niger, and kept under sterile conditions until the fruits were decayed, or, in the case of the Penicillium and Aspergillus, until partially decayed. The decayed fruits were then digested with hydrochloric acid and analyzed for their oxalic acid content with the following results:

Peach inoculated with Penicillis	um No trace of oxalic acid,
Peach inoculated with Aspergill	us No trace of oxalic acid,
Peach inoculated with Sclerotini	a cinerea
	0.0087 grams of oxalic acid,
Deach control	No trace of evalia said

The results of these experiments with oxalic acid show that Sclerotinia cinerea when grown either on fruit juices or on peaches produces more or less oxalic acid as a result of its metabolism. It is also significant that the other two fungi employed, namely, Aspergillus and Penicillium, which are not natural parasites on the plum or the peach, produced no oxalic acid under the conditions in which the experiments were carried out.

#### SUMMARY

1. The brown-rot organism will infect fruits which are immature, even penetrating those which are not more than half-grown or those in which the pits are still soft, provided the

skin is punctured. Infection of green fruits is also effected when a portion of the mycelial felt of the fungus is laid on the surface of the plum. On the other hand, ripe or nearly mature fruits may be readily inoculated by sowing a spore suspension on the unpunctured surface.

2. The fungus does not show any particular affinity for the middle lamella, but penetrates and permeates with equal avidity

any part of the host tissue.

3. A study of the effect of the organism on the host gives no positive evidence that a toxic substance is abundantly secreted in advance of penetration.

4. The fungus shows very slight cytolytic action with respect to cellulose isolated from the plum, while, on the other hand, the organism readily hydrolyzes cellulose from filter paper when this is the only carbohydrate supplied. No general cytolytic action of the organism on the cell wall of the host is perceptible.

- 5. An aqueous solution of pectin isolated from plums was coagulated by *Sclerotinia*, thus indicating the secretion of the enzyme pectinase. In respect to its action on pectic substances, *Sclerotinia cinerea* behaves in a manner similar to that of *Penicillium expansum*, yet these two organisms produce very different effects on the host, the former producing a firm rot and the latter a soft one. Neither organism will dissolve calcium pectinate.
- 6. The experiments on the acid relations of the fungus indicate that the changing acidity of the host as the fruit reaches maturity does not explain the fact that ripe fruit is more susceptible to the disease than green fruit.
- 7. The brown-rot fungus produces oxalic acid when grown either on a fruit juice medium or on peaches.

The writer takes pleasure in acknowledging his indebtedness to Professor B. M. Duggar for his advice and helpful criticism in this investigation. Part of this work was done during the summer of 1913 in the Laboratory of Plant Pathology of the University of Wisconsin, and the writer wishes to express his gratitude to Professor L. R. Jones for the courtesy extended to him while at Madison.

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#### BIBLIOGRAPHY

- de Bary, A. Recherches sur le développement de quelques champignons parasites. Ann. d. Sci. Nat. Bot. IV. 20: 5-148. 1863.
- Neue Untersuchung ueber Uredineen II. Monatsber. d. Akad. d. Wiss. z. Berlin. 1886.
- Morphologie und Physiologie der Pilze, Flechten, und Myxomyceten. Handbuch der physiologischen Botanik 2: 1-316. 1866. [cf. pp. 212-19.]
- Ueber einige Sclerotinien und Sclerotienkrankheiten. Bot. Zeit. 44: 377-87, 393-404, 409-26, 433-41, 449-61, 465-74. 1886.
- Behrens, J. Untersuchungen über die Gewinnung der Hanffaser durch natürliche Röstmethoden. Centralbl. f. Bakt. II. 8: 114-20, 131-37, 161-66, 202-10, 231-36, 264-68, 295-99. 1902.
- Beiträge zur Kenntnis der Obstfäulnis. Centralbl. f. Bakt. II. 4: 514-22, 547-53, 577-85, 635-44, 700-706, 739-46, 770-77. 1898.
- Bertrand, G., et Mallèvre, A. Recherches sur la pectase et sur la fermentation pectique. I. Jour. de Bot. 8: 390-96. 1894.
- , ——, Sur la diffusion de la pectase dans le règne végétal et sur la préparation de cette diastase. Jour. de Bot. 10: 37-41. 1896.
- Biffen, R. H. On the biology of Bulgaria polymorpha, Wett. Ann. Bot. 15: 119-34. pl. 7. 1901.
- Bigelow, W. D., Gore, H. C., and Howard, B. J. Studies on apples. U. S. Dept. Agr., Bur. Chem. Bul. 94: 1-99. pl. 1-5, 1905.
- Bourquelot, Em. Sur la physiologie du gentianose; son dédoublement par les ferments solubles. Compt. rend. acad. Paris 126: 1045-47. 1898.
- ------, et Hérissey, H. Sur l'existence dans l'orge germée d'un ferment soluble agissant sur la pectine. Compt. rend. acad. Paris 127: 191-94. 1898.
- , \_\_\_\_\_\_\_\_, Les ferments solubles du Polyporus sulfureus (Bull.). Bull. Soc. Myc. Fr. 11: 235–39. 1895.
- Buller, A. H. R. The enzymes of Polyporus squamosus, Huds. Ann. Bot. 20: 49-59. 1906.
- The destruction of wood paving blocks by Lentinus lepideus. Fr. Jour. Econ. Biol. 1: 1-10. pl. 1-11. 1905.
- Büsgen, M. Ueber einige Eigenschaften der Keimlinge parasitischer Pilze.
   Bot. Zeit. 5x<sup>1</sup>: 53-72. pl. 2-3. 1893.
- Cook, M. T., Bassett, H. T., Thompson, F., and Taubenhaus, J. J. Protective enzymes. Science N. S. 33: 624-29. 1911.
- Czapek, F. Zur Biologie der holzbewohnenden Pilze. Ber. d. deut. bot. Ges. 17: 166-70. 1899.
- Dox, A. W. The intracellular enzyms of Penicillium and Aspergillus. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 120: 1-70. 1910.
- Emmerling, O. Oxalsäurebildung durch Schimmelpilze. Centralbl. f. Bakt II. 10: 273-75. 1903.
- 21. Euler, H. General chemistry of ensymes. 1-319. 1912.
- Falck, R. Hausschwamforschungen. Zeitschr. f. Forst. u. Jagdwesen. Heft
   p. 1–405. pl. 1–17. 1912. [cf. pp. 273–80.]
- Fremey, E. Premiers essais sur la maturation des fruits. Recherches sur la pectine et l'acide pectique. Jour. de Pharmacie 26: 368-93. 1840. [Original not consulted.]

- Fremey, E. Memoire sur la maturation des fruits. Ann. Chim. et Phys. III.
   1-58, 1848. [Original not consulted.]
- Fulton, H. R. Chemotropism of fungi. Bot. Gaz. 41: 81-108. 1906.
- Hartig, R. Die Zersetzungserscheinungen des Holzes der Nadelholzbäume und der Eiche. Berlin. 1–151. pl. 1–21. 1878.
- 27. Humphrey, J. E. On Monilia fructigena. Bot. Gaz. 18: 85-93. pl. 7. 1893.
- Van Iterson, C., Jr. Die Zersetzung von Cellulose durch aërobe Mikroorganismen. Centralbl. f. Bakt. II. 11: 689–98. pl. 1. 1904.
- Jones, L. R. The cytolytic enzyme produced by Bacillus carotovorus and certain other soft rot bacteria. Centralbl. f. Bakt. II. 14: 257-72. 1905.
- Harding, H. A., and Morse, W. J. The bacterial soft rots of certain vegetables. I. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bul. 11: 251–368. 1909. [cf. pp. 291–368.] *Ibid*, Vermont Agr. Exp. Sta. Bul. 147: 243–360. 1910. [cf. pp. 283–360.]
- Kellerman, K. F. The excretion of cytase by Penicillium Pinophilum. U. S. Dept. Agr., Bur. Pl. Ind. Circ. 118: 29-31. 1913.
- ——, and McBeth, I. G. The fermentation of cellulose. Centralbl. f. Bakt. II. 34: 485-94. pl. 1-2. 1912.
- Kohnstamm, P. Amylolytische, glycosidspaltende, proteolytische und cellulose lösende Fermente in holzbewohnenden Pilzen. Beih. z. bot. Centralbl. 10: 90-121. 1901.
- Kühn, J. Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung. Berlin. 1–312. pl. 1–7. 1858.
- Mangin, L. Propriétés et réactions des composés pectiques. Jour. de Bot. 6: 206-12, 235-44, 363-68. 1892.
- Recherches sur les composés pectiques. Jour. de Bot. 7: 37-47. pl. 1., 121-31. pl. 2., 325-43. 1893.
- Matheny, W. A. A comparison of the American brown-rot fungus with Sclerotinia fructigena and S. cinerea of Europe. Bot. Gaz. 56: 418-32. f. 1-6. 1913.
- McBeth, I. G., and Scales, F. M. The destruction of cellulose by bacteria and filamentous fungi. .U. S. Dept. Agr., Bur. Pl. Ind. Bul. 266: 1-52. pl. 1-4. 1913.
- Miyoshi, M. Die Durchbohrung von Membranen durch Pilzfäden. Jahrb. f. wiss. Bot. 28: 269-89. 1895.
- Nordhausen, M. Beiträge zur Biologie parasitärer Pilze. Jahrb. f. wiss. Bot. 33: 1-46. 1899.
- Peltier, G. L. A consideration of the physiology and life history of a parasitic Botrytis on pepper and lettuce. Rept. Mo. Bot. Gard. 23: 41-74. pl. 1-5. 1912.
- Reed, H. S. The enzyme activities involved in certain fruit diseases. Ann. Rept. Va. Agr. Exp. Sta. 1911-1912; 51-77. 1912.
- Schellenberg, H. C. Untersuchungen über das Verhalten einiger Pilze gegen Hemizellulosen. Flora 98: 257–308. 1908.
   Schmidt, E. W. Über den Parasitismus der Pilze. Zeitschr. f. Pflanzenkrankh.
- Schmidt, E. W. Uber den Parasitismus der Pilze. Zeitschr. f. Pflanzenkrankh. 19: 129–43. 1909.
- Smith, E. F. Peach rot and peach blight. (Monilia fructigena Pers.) Jour. Myc. 5: 123-34. 1899.
- 46. Smith, R. E. The parasitism of Botrytis cinerea. Bot. Gas. 33: 421-36. 1902.
- von Schrenk, H. A disease of the black locust (Robinia pseudacacia L.). Rept. Mo. Bot. Gard. 12: 21-31. pl. 1-3. 1901.

- Thom, C. Cultural studies of species of Penicillium. U. S. Dept. Agr., Bur. Animal Ind. Bul. 118: 1-109. f. 1-36. 1910.
- Thompson, F., and Whittier, A. C. Fruit juices. Del. Agr. Exp. Sta. Bul. 102: 1-28. 1913.
- Ward, H. M. On the biology of Stereum hirsutum. Phil. Trans. Roy. Soc. Lond. 189: 123-34. 1897.
- 51. ——, A lily-disease. Ann. Bot. 2: 319-82. pl. 20-24. 1888.
- 52. ———, Penicillium as a wood-destroying fungus. Ann. Bot. 12: 565-66. 1898.
- Wehmer, C. Monilia fructigena Pers. (=Sclerotinia fructigena m) und die Monilia-Krankheit der Obstbäume. Ber. d. deut. bot. Ges. 16: 298-307. pl. 18. 1898.
- 54. \_\_\_\_\_\_\_, Entstehung und physiologische Bedeutung der Oxalsäure im Stoffwechsel einiger Pilze. Bot. Zeit. 49: 233-46, 249-57, 271-80, 289-98, 305-13, 321-32, 337-46, 353-63, 369-74, 385-96, 401-7, 417-28, 433-39, 449-56, 465-78, 511-18, 531-39, 547-54, 563-69, 579-84, 596-602, 611-20, 630-38. 1891.
- Winogradsky, S. Sur le rouissage du lin et son agent microbien. Compt. rend. acad. Paris 121: 742-45. 1895.
- Woronin, M. Über Sclerotinia cinerea und Sclerotinia fructigena. Mem. de l'Acad. Imp. d. Sci. de St. Petersbourg, Classe Phys. Math. VIII. 10: 1-38. pl. 1-6. 1899.
- Zschokke, A. Ueber den Bau der Haut und die Ursachen der verschwindenen Haltbarkeit unserer Kernobstfrüchte. Landw. Jahrb. d. Schweiz 11: 153-97. pl. 1-2. 1897.

# THE THELEPHORACEÆ OF NORTH AMERICA. II1

## CRATERELLUS

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#### CRATERELLUS

Craterellus Pers. Myc. Eur. 2:4. 1825.—Fries, Epicr. 531. 1838; Hym. Eur. 630. 1874.—Saccardo, Syll. Fung. 6:514. 1888.—Hennings, in Engl. & Prantl, Nat. Pflanzenfam. (1. 1\*\*): 127. 1898.

The type species of the genus is Craterellus cornucopioides L. ex Pers.

Fructifications fleshy or membranaceous, pileate, often tubiform, infundibuliform, or flabelliform, sometimes clavate; hymenium waxy-membranous, distinct, continuous, adnate to the hymenophore, even or rugose; basidia simple; spores usually white.

Craterellus is closely related by its fleshy C. Cantharellus, C. odoratus, C. lutescens, etc., with the genus Cantharellus. These species resemble so closely in coloration and habit species of the latter genus that careful examination of the hymenium should be made for generic determination. Craterellus has its hymenium even or slightly rugose. In exceptional connecting species, such as C. clavatus, it is somewhat lamelliform for a part of the distance from margin of the pileus to the stem. The clavate C. pistillaris and C. unicolor connect Craterellus closely with Clavaria.

Craterellus cornucopioides, C. ochrosporus, C. clavatus, C. Cantharellus, and C. odoratus are edible species, which are often abundant locally.

<sup>1</sup> Issued September 30, 1914.

NOTE.—Explanation in regard to the citation of specimens studied is given in Part I, Ann. Mo. Bot. Gard. 1: 202, footnote. The technical color terms used in this work are those of Ridgway, Color Standards and Nomenclature. Washington, D. C., 1912.

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# KEY TO THE SPECIES

Hymenium somewhat radiately lamelliform—at least near the margin;
stem solid
Hymenium plane, rugose-wrinkled, or ribbed and rugose-wrinkled 2
1. Fructification large, 4-10 cm. high; stem about 1 cm. thick; spores 10-13 x 4-4½ \(\mu\)
1. Fructification small, 1-1\frac{1}{2} cm. high; stem 1 mm. thick; pileus umbilicate;
spores 9 x 7 μ
2. Fructification with pileus infundibuliform and pallid rose; hymenium
and stem white. In N. Carolina in moss near Kalmia bushes4. C. roseus
2. Fructification entirely egg-yellow, about 3-9 cm. high, 21-9 cm. broad 3
2. Fructification neither entirely egg-yellow nor with pileus pallid rose and hymenium and stem white
3. Pileus convex, then depressed or infundibuliform; stem solid 2. C. Cantharellus
3. Pileus convex, then depressed or cyathiform; stem hollow or cavernous;
fructification sometimes branched
the stem
4. Pileus not tubiform, but instead infundibuliform, depressed, truncate,
convex, or flabelliform
5. Pileus and stem smoky brown to blackish; hymenium cinereous drab; spores
12-16 x 6-10 μ
<ol><li>Pileus drying avellaneous to snuff-brown; stem black with chamois-colored pubescence at its base; hymenium chamois-colored or colored like the pileus;</li></ol>
spores 12–15 x 7–8 $\mu$
<ol> <li>Pileus somewhat tubiform; hymenium dark cinereous; spores 6-7½ x 4½-5 μ</li> </ol>
7. C. dubius
5. Pileus somewhat tubiform or umbilicate, yellowish brown to fuscous; hyme-
nium and stem yellow; spores $10-12 \times 6-8 \mu$
spores 10-12 x 6-7 $\mu$
6. Pileus deeply cup-shaped, 4-8 mm. broad; hymenium cream-buff;
spores 8 x 6 μ
6. Pileus convex, then umbilicate, 5 mm. broad; hymenium sometimes
obscurely lamelliform, chamois-colored; stem chamois-colored; spores
9 x 7 \( \therefore \) 11. C. deltiescens 6. Pileus merely depressed, truncate, convex, or clavate
6. Pileus flabelliform
7. Fructification small, 1-3 cm. high, 4-9 mm. broad, narrowly obconic, white;
spores 3-4 \mu in diameter
7. Fructification 2-5 cm. high, from obconic often becoming abruptly enlarged
and somewhat cerebriform at the upper end but with the stem remaining comparatively slender
7. Fructification large, 6-15 cm. high, clavate or obconic and truncate, tapering
downward; stem often bulbous at the base. Fructification dries sorghum-
brown to fuscous
8. Pileus ligulate at first, then spreading laterally and becoming somewhat
palmately cleft into a few branches, fawn-color shading into bone-
brown. Known from Ohio

 Pileus somewhat triangular, drying a dirty pinkish buff; hymenium drying Isabella-color to clay-color. Known only from Florida. 16. C. dilatus

8. Fructification entirely white; pileus reniform, dimidiate, attached laterally to a slender erect stem. Known only from Washington

17. C. Humphreyi

r. Craterellus clavatus Pers. ex Fries, Epicr. 533. 1836-1838. Plate 15. fig. 6.

Merulius clavatus Pers. Obs. Myc. 1: 21. 1896.—Cantharellus clavatus Fries, Syst. Myc. 1: 322. 1821.—Nevrophyllum clavatum Fries ex Patouillard, Tab. Anal. Fung. 1: 193. f. 434. 1883–1886.—Cantharellus brevipes Peck, Rep. N. Y. State Mus. 33: 21. pl. 1. f. 18-20. 1879.

Illustrations: Schæffer, Icon. Fung. pl. 164, 276.—Krombholz, Abbild. und Beschr. pl. 45. f. 13-17.—Fries, Sverig. Ätl. Svamp. pl. 91.—Richon et Roze, Atlas Champ. pl. 50. f. 10-14.—Bresadola, Funghi Manger. pl. 82.—Peck, Rep. N. Y. State Mus. 33: pl. 1. f. 18-20.—Harper, Mycologia 5: pl. 93, 94.

Fructifications solitary or cespitose, fleshy, flesh whitish; pileus narrowly obconic, turbinate, truncate or depressed, glabrous, ochraceous buff, attenuated into the stem, the margin thin and erect; stem short, solid, tomentose at the base; hymenium lamelliform near the margin, rugose-wrinkled elsewhere, becoming pruinose with the spores, light vinaceous drab, drying drab; spores pale ochraceous in the mass, 10-13 x 4-4½  $\mu$ .

Fructifications 4-10 cm. high; pileus 3-8 cm. broad; stem 1-2 cm. long, 8-15 mm. thick.

On the ground in coniferous woods. Maine to Connecticut and west to Minnesota, and in Montana. July to September.

This species is intermediate between Craterellus and Cantharellus. The marginal portion of the hymenium is like that of a Cantharellus, and the remainder of the hymenium, like that of a Craterellus. There is good authority for including this species in Cantharellus and there is the authority of Fries and herbarium usage for classing it in Craterellus. C. clavatus is edible but too rare, at least in the east, to be common in herbaria.

Specimens examined:

Exsiccati: De Thuemen, Myc. Univ., 1807.

Austria: G. Bresadola.

Maine: Sprague (in Curtis Herb., 5786).

New Hampshire: Shelburne, W. G. Farlow (in Mo. Bot. Gard. Herb., 4868).

Vermont: Lake Dunmore, E. A. Burt.

Connecticut: Rainbow, C. C. Hanmer, 1454 (in Hanmer Herb.). New York: Ballston, C. H. Peck, the type of Cantharellus brevipes (in Coll. N. Y. State).

 C. Cantharellus Schw. ex Fries, Epicr. 534. 1836–1838. Plate 15. fig. 7.

Thelephora Cantharella Schw. Schrift. d. Naturforsch. Gesell., Leipzig, 1: 105. 1822.—Craterellus lateritius Berk. Grevillea 1: 147. 1873.

Illustrations: Peck, Rep. N. Y. State Mus. 49: pl. 44. f. 1-5; Mem. N. Y. State Mus. 3<sup>4</sup>: pl. 56. f. 17-21.—Hard, Mushrooms f. 378.—Marshall, Mushroom Book 73. f.

Type: in Herb. Schweinitz.

Fructifications single or cespitose, fleshy, firm, egg-yellow; pileus convex, becoming depressed or infundibuliform, glabrous, yellow, the margin often lobed or irregular; stem solid, cylindric or tapering downward, glabrous, yellow; hymenium nearly even or rugose wrinkled, yellow, or with a reddish salmon tinge and drying ochre-red; spores  $7-10 \times 3\frac{1}{2}-5\frac{1}{2} \mu$ .

Fructifications 4-9 cm. high; pileus 2½-8 cm. broad; stem

 $2\frac{1}{2}$ -5 cm. long, 5-10 mm. thick.

On the ground in open woods. Massachusetts to Alabama and westward to Ohio; also in Mexico. June to September. Abundant locally.

This species is so similar to Cantharellus cibarius in habit, coloration, size and form—differing from the latter only in the more even hymenium, that figures of C. cibarius will serve very well for Craterellus Cantharellus, if allowance is made for the different hymenium. The firm and solid stem of C. Cantharellus distinguishes this species from C. odoratus easily. The latter species sometimes has its pileus greatly branched. My illustration of this species is photographed from the dried herbarium specimen of the cotype of C. lateritius Berk. In this specimen the lobes of the pileus were pressed together above before drying. The hymenium of this specimen is now ochre-red and agrees in color with that of the authentic specimen of C. Cantharellus in Curtis Herb.; both these specimens have been poisoned. I

found the spores of the type in Herb. Schw. 8-9 x  $3\frac{1}{2}$ -4  $\mu$ , or a little slenderer than in northern specimens. Hard states that the spores are yellowish or salmon colored in the mass when collected. This species is edible.

Specimens examined:

Exsiccati: Ell. & Ev., N. Am. Fungi, 1921.

Massachusetts: Sprague (in Curtis Herb.); Milton, H. Webster. Connecticut: East Hartford, C. C. Hanner, 2391, 2468 (both in Hanner Herb.).

Pennsylvania: West Chester, B. M. Everhart, Ell. & Ev., N. Am. Fungi, 1921.

West Virginia: Eglon, C. G. Lloyd, 02292.

North Carolina: Schweinitz, type (in Herb. Schweinitz); Blowing Rock, G. F. Atkinson, 4313.

South Carolina: Clemson College, P. H. Rolfs, 1830.

Alabama: Peters (in Curtis Herb., 4539, and in Kew Herb.), the cotype and type respectively of C. lateritius; Auburn, F. S. Earle (in Mo. Bot. Gard. Herb., 4928).

Ohio: A. P. Morgan (in Lloyd Herb.). Kentucky: C. G. Lloyd (in Lloyd Herb.).

Mexico (?): Botteri, 27 (in Curtis Herb.). If the stem is hollow this specimen is C. odoratus.

3. C. odoratus Schw. ex Fries, Epicr. 532. 1836-1838.

Plates 15, 16. figs. 8-10.

Merulius odoratus Schw. Schrift. d. Naturforsch. Gesell., Leipzig, 1: 91. 1822.—Cantharellus odoratus Fries, Elenchus Fung. 1: 51. 1828.—C. confluens Berk. & Curtis, Jour. Linn. Soc. Bot. 9: 423. 1867.

Type: in Herb. Schweinitz.

Fructifications gregarious, sometimes cespitose, simple or branched, egg-yellow; pileus thin, convex, then depressed and somewhat cyathiform, sometimes pervious, yellow, the margin deflexed, often lobed or irregular; stem cylindric or somewhat tapering towards the base, concolorous with the pileus, hollow or cavernous; hymenium even or somewhat rugose-wrinkled, ochraceous orange or with a reddish tinge approaching Sanford's brown; spores even,  $7-9 \times 4-5 \mu$ .

Fructifications 3-7 cm. high; pileus 2-9 cm. broad; stem 2-4 cm. long, 3-8 mm. thick.

In moist places in woods. North Carolina and Georgia to Ohio and Missouri. June to October.

Specimens of this species have sometimes been confused in recent years with the better known C. Cantharellus, which ranges farther north. The color and general habit of these species is the same; both have the egg-vellow color and the characteristic fragrance of Cantharellus cibarius when moistened after drying. and all three are edible. Craterellus odoratus is more membranaceous than C. Cantharellus and it differs from both this species and Cantharellus cibarius in having a hollow or cavernous stem whose pliant walls may be pinched together, like those of a rubber tube, before the specimens are dried. Highly branched forms may occur as shown in pl. 16 fig. 10a; this character was unduly emphasized in the original description. The ample collections in the Glatfelter Herbarium seem to show that Craterellus odoratus is the most frequent Craterellus in the vicinity of St. Louis. Dr. Glatfelter notes on his collection that he has eaten this species and found it quite good. In pl. 15 fig. 8, I give a figure, natural size, from a photograph of the dried herbarium cotype of C. confluens B. & C., to show how close the resemblance is to the specimens of C. odoratus, collected at St. Louis and figured in the following plate. The type of C. confluens has the hymenium rugose-wrinkled, as is often the case in specimens of C. odoratus; its habit, dimensions, structure, coloration, and spores are quite those of C. odoratus.

Specimens examined:

North Carolina: Salem, Schweinitz, type (in Herb. Schweinitz). South Carolina: Society Hill, Ravenel, 192 (in Curtis Herb.).

Georgia: Station cited by Schweinitz. Alabama: Auburn, L. M. Underwood.

Ohio: Oxford, L. O. Overholts, 1721 (in Overholts Herb.).

Missouri: near St. Louis, N. M. Glatfelter, 348 (in Mo. Bot. Gard. Herb., 42590), and J. B. S. Norton (in Mo. Bot. Gard. Herb., 4926).

Mexico: near Orizaba, Botteri, 6 (type and cotype in Kew Herb. and Curtis Herb., respectively, of C. confluens).

4. C. roseus Schw. ex Fries, Epicr. 533. 1836-1838.

Merulius roseus Schw. Schrift. d. Naturforsch. Gesell., Leip-

zig, 1: 91. 1822.—Cantharellus roseus Fries, Elenchus Fung. 53. 1828.

Fructifications solitary, somewhat fleshy; pileus infundibuliform, somewhat strigose, pallid rose, the margin lobed and inflexed; stem apparently stuffed, attenuated downward, white; hymenium somewhat rugose, white.

In mosses, especially in proximity to Kalmia. North Carolina. Specimens of this species have the habit of Cantharellus cibarius but are thinner. Fries received a specimen of Craterellus roseus from Schweinitz and expressed the opinion in 'Elenchus' that the species is good. I have seen no specimens of C. roseus and base the above on the original description and the comments by Schweinitz and Fries.

### 5. C. cornucopioides L. ex Pers. Myc. Eur. 2: 5. 1825.

Plate 17, fig. 17.

Peziza cornucopioides L. Sp. Pl. 1181. 1753. [1st ed.]— Elvella cornucopioides Scop. Fl. Carn. 2: 476. 1760.—Merulius cornucopioides Pers. Syn. Fung. 491. 1801.—Cantharellus cornucopioides Fries, Syst. Myc. 1: 321. 1821.

Illustrations: Vaillant, Botan. Paris. pl. 13. f. 2, 3.—Bolton, Hist. Fung. pl. 103.—Flor. Dan. pl. 384, 1260.—Holmskiold, Fung. Dan. 2. pl. 5.—Sowerby, Brit. Fung. pl. 74.—Schæffer, Icon. Fung. pl. 165.—Bulliard, Herb. de la France pl. 150.—Schnizlein, in Sturm, Deutsch. Flora 3: fasc. 31. pl. 5.—Bresadola, Funghi Manger. 75. pl. 83.—Cooke, Brit. Edible Fung. pl. 11. f. 39.—Dufour, Atlas Champ. pl. 70. f. 157.—Hard, Mushrooms 451. f. 379.—Peck, Rep. N. Y. State Mus. 48: pl. 24. f. 7-10.—cf. Saccardo, Syll. Fung. 19: 478, for other references to illustrations.

Fructifications gregarious or somewhat cespitose; pileus thin, somewhat membranaceous, tubæform, pervious, sometimes granular or minutely squamulose, smoky brown to blackish, usually drying Prout's brown, with the erect, spreading, or decurved margin generally lobed, wavy, or irregular; stem short, hollow, even, blackish brown; hymenium even or rugosewrinkled, cinereous drab; spores hyaline, even,  $12-16 \times 6-10 \mu$ .

Fructification 5-8 cm. high; pileus  $2\frac{1}{2}$ -5 cm. broad; stem 1-3 cm. long, 3-5 mm. thick.

On earth in mixed woods. Canada to South Carolina and westward to Missouri. June to September.

The cornucopia craterellus is well characterized by its cornucopia-shaped or narrowly trumpet-shaped pileus ashy to sooty brown in color, by thin flesh which is somewhat tough and flexile, cinereous drab hymenium which sometimes has a brownish tinge, and black stem. This species is too infrequent to afford more than a few herbarium specimens in the regions where I have collected fungi, but it is reported so plentiful in some states as to be highly regarded as an edible species.

Specimens examined:

Exsiccati: Ravenel, Fung. Car. II. 27; Ellis, N. Am. Fungi, 321; Ell. & Ev., Fung. Col., 1723; Shear, N. Y. Fungi, 49; Rabenhorst-Winter, Fung. Eur., 3640.

Sweden: L. Romell, 48. Canada: J. Macoun, 72, 73.

Ontario: Casselman, J. Macoun, 347. Vermont: Grand View Mt., E. A. Burt.

Massachusetts: Sprague, 211 (in Curtis Herb.).

Connecticut: W. A. Setchell.

New York: Sand Lake, C. H. Peck (in Coll. N. Y. State); Alcove, C. L. Shear, Shear's N. Y. Fungi, 49; Ithaca, H. von Schrenk (in Mo. Bot. Gard. Herb., 4763, 42584), W. H. Long, Jr., Ell. & Ev., Fung. Col., 1723.

New Jersey: Newfield, H. Leahy, Ellis, N. Am. Fungi, 321. Pennsylvania: locality cited by Schweinitz, Syn. N. Am.

Fungi; W. Herbst (in Lloyd Herb.).

North Carolina: (in Curtis Herb., 502); locality cited by Schweinitz, Syn. Fung. Car. Sup.

South Carolina: M. A. Curtis (in Curtis Herb.).

Ohio: Loveland, D. L. James, comm. by U. S. Dept. Agr.

Kentucky: Mammoth Cave, C. G. Lloyd.

Missouri: Perryville, C. H. Demetrio, Rabenhorst-Winter, Fung. Eur., 3640; Meramec Highlands, P. Spaulding (in Mo. Bot. Gard. Herb., 4869).

6. C. ochrosporus Burt, n. sp. Plate 17. fig. 15. An C. ocreatus Pers. Myc. Eur. 2: 5. pl. 13. f. 2. 1825?

Type: in Mo. Bot. Gard. Herb., 42585.

Fructifications gregarious or cespitose; pileus thin, somewhat

membranaceous, tubæform, pervious, minutely floccose-squamulose, drying avellaneous to snuff-brown, the margin erect or decurved; stem short, hollow, black, with chamois-colored pubescence at the base; hymenium even or somewhat rugose, sometimes colored like the pileus but in the type chamois-colored; spores straw-yellow in the mass, even, obtuse,  $12-15 \times 7-8 \mu$ .

Fructifications 4-7 cm. high; pileus  $1-3\frac{1}{2}$  cm. broad,  $1-2\frac{1}{2}$  cm. long, 2-4 mm. thick.

On the ground among mosses in woods. New York and Missouri. June to September. Probably abundant in Missouri.

Dr. Glatfelter noted a pleasant minty odor for the specimens. This species closely resembles C. cornucopioides in form, but differs from that species in having hymenium, spores, and base of stem yellow. A collection from the same spot from which the type collection came, but made in June two years later, has the hymenium snuff-brown and approaches C. cornucopioides in this respect. I am not aware of any data on C. ocreatus Pers. except that based on the original description which is cited above. That species has presumably not been collected by European mycologists since the original collection from the environs of Paris a century ago. Our specimens differ from that description in having the stem yellow pubescent at the base and the hymenium somewhat rugose, and they may differ in other characters, e. g., spore colors, etc., not given in the brief description of C. ocreatus. Hence I give to our American specimens a distinct name.

Specimens examined:

New York: East Galway, E. A. Burt.

Missouri: Meramec Highlands, N. M. Glatfelter (in Mo. Bot. Gard. Herb., 42585, type, and 42586-87); Columbia, B. M. Duggar, 134.

7. C. dubius Peck, Rep. N. Y. State Mus. 31: 38. 1879.

Illustrations: Hard, Mushrooms f. 380.

Type: in Coll. New York State.

Fructifications solitary or cespitose; pileus thin, infundibuliform or subtubiform, subfibrillose, dark brown or lurid brown, pervious, the margin generally wavy and lobed; stem short, hollow, colored like the hymenium; hymenium dark cinereous and rugose when moist, the obscure crowded irregular wrinkles abundantly anastomosing, nearly even and paler when dry; spores broadly elliptical or subglobose,  $6-7\frac{1}{2} \times 4\frac{1}{2}-5 \mu$ .

Fructification  $5-7\frac{1}{2}$  cm. high; pileus  $2\frac{1}{2}-5$  cm. broad, 4 mm.

thick.

On ground in woods. Ontario and New York to Illinois. August to October. Rare.

The specimens of this species have the same coloration as those of *C. cornucopioides* but differ from the latter in having a shorter and more funnel-shaped pileus, and smaller spores. Moffatt reported *C. dubius* as abundant at Glencoe, Illinois.

Specimens examined:

Ontario: Belleville, J. Macoun, 228 (in Coll. N. Y. State). New York: Adirondack Mts., C. H. Peck, type (in Coll. N. Y. State).

Michigan: Sailor's Encampment, Univ. of Wis. Herb., 46.

8. C. lutescens Pers. ex Fries, Epicr. 532. 1838.

Plate 17. fig. 20.

Merulius lutescens Pers. Syn. Fung. 489. 1801; Albertini & Schweinitz, Consp. Fung. 234. 1805.—Cantharellus lutescens Fries, Syst. Myc. 1: 320. 1821.—Merulius xanthopus Pers. Myc. Eur. 2: 19. pl. 13. f. 1. 1825.

Illustrations: Vaillant, Botan. Paris. pl. 11. f. 9, 10.—Schæffer, Icon. Fung. pl. 157.—Bolton, Hist. Fung. pl. 105. f. 2.—Persoon, Myc. Eur. 2: pl. 13. f. 1.—Hennings, in Engl. & Prantl, Nat. Pflanzenfam. (1.1\*\*): 129. f. 70 H.—Stevenson, Brit. Hym. 2: 259.

Fructifications solitary to cespitose; pileus thin, somewhat membranaceous, varying from convex and umbilicate to tubiform or funnel-shaped, often pervious, yellowish brown to fuscous, with margin often lobed or irregular; stem flexuous, cylindric, hollow, yellow, drying ochraceous buff, often hairy at the base; hymenium remotely ribbed, even or rugose-wrinkled, yellow, drying cadmium-yellow to ochraceous buff; spores even,  $10-12 \times 6-8 \mu$ .

Fructifications  $2\frac{1}{2}$ -5 cm. high; pileus  $1\frac{1}{2}$ -3 cm. broad, stem  $1\frac{1}{2}$ -4 cm. long, 2-4 mm. thick.

On moist ground in woods and swamps. Newfoundland to North Carolina and westward to Michigan. August to October.

n

This species probably ranks next to *C. cornucopioides* in frequency in the United States. The long and yellow stem readily distinguishes this species from *C. ochrosporus*. Specimens of *Cantharellus infundibuliformis* resemble those of *Craterellus lutescens* in form, size, and color, but those of the former species have true lamellæ.

Specimens examined:

Exsiccati: Ellis, N. Am. Fungi, 1302; De Thuemen, Myc. Univ., 404.

Sweden: Stockholm, L. Romell, 49; Femsjö, L. Romell.

Newfoundland: Bay of Islands, A. C. Waghorne, 34 (in Mo. Bot. Gard. Herb.).

New Hampshire: Shelburne, W. G. Farlow, Ellis, N. Am. Fungi, 1302, and (in Mo. Bot. Gard. Herb., 4932).

Vermont: Lake Dunmore, E. A. Burt.

Massachusetts: Worcester, G. E. Francis, 100. New England: Sprague, 1689 (in Curtis Herb.).

New York: Sand Lake and Helderberg Mts., C. H. Peck (in Coll. N. Y. State); East Galway, E. A. Burt.

Pennsylvania: locality cited by Schweinitz, Syn. N. Am. Fungi. North Carolina: locality cited by Schweinitz, Syn. Fung. Car. Sup.

Michigan: Glen Lake, C. G. Lloyd, 02462.

9. C. sinuosus Fries ex Fries, Epicr. 533. 1836–1838.

Cantharellus sinuosus Fries, Syst. Myc. 1:319. 1821.
Illustrations: Vaillant, Botan. Paris. pl. 11. f. 11-23.—Fries, Icon. Hym. 2: pl. 196. f. 2.—Dangeard, Le Botaniste 4:147. f.—Gillet, Champ. France Hym. pl.

Fructifications cespitose, slightly fleshy; pileus infundibuliform, undulate and floccose, light drab; stem cylindric, stuffed, pallid cinereous; hymenium at length with interwoven wrinkles, pallid cinereous; spores  $10-12 \times 6-7 \mu$ .

Fructifications 2-3 cm. high; pileus 2-3 cm. broad; stem  $1\frac{1}{2}$ -2 cm. long, 2-4 mm. thick.

On ground in mixed woods. South Carolina. Rare.

I have seen only dried herbarium specimens of Craterellus sinuosus. The spore measurements are those of a specimen from Sweden received from Romell. In this specimen the hymenium has dried somewhat chamois-colored.

Specimens examined:

Exsiccati: Rabenhorst, Fung. Eur., 208 (in Kew Herb.).

Sweden: L. Romell, 50.

South Carolina: Ravenel (in Curtis Herb., 2982).

C. crispus Fr., sometimes regarded as a variety of C. sinuosus, was reported from New England, Sprague, by Berkeley & Curtis, Grevillea 1: 147, but the specimen is not satisfactory for study. I do not, therefore, like to include C. crispus as one of our species.

10. C. calyculus (B. & C.) Burt, n. comb.

Stereum calyculus Berk. & Curtis, Hooker's Jour. Bot. and Kew Gard. Misc. 1: 238. 1849; Grevillea 1: 161. 1873.

Type: type and cotype in Kew Herb. and Curtis Herb. respectively.

Fructifications somewhat fleshy-membranaceous; pileus thin, deeply cup-shaped, minutely tomentose, drying Saccardo's umber, opaque; stem apparently hollow, cream buff, attenuated below, tomentose at the base; hymenium even or slightly venose, cream buff; spores slightly yellowish under the microscope, even,  $8 \times 6 \mu$ .

Fructifications 2-3 cm. high; pileus 4-8 mm. broad; stem 1 cm. long, 1-2 mm. thick.

On ground in damp shady woods. North and South Carolina. August and September.

Upon moistening, the type in Kew Herbarium proved too soft and fleshy and the hymenium too waxy for a Stereum. The sections have the structure of Craterellus. The species is near C. sinuosus and may prove to be a small form of this when ample material gives more complete knowledge of the species, but, for the present, I regard C. calyculus as a distinct species. I refer to C. calyculus a collection made by Professor Atkinson at Blowing Rock, North Carolina, the rough-dried and cespitose specimens of which show a somewhat tubiform pileus and spores  $7-8 \times 4\frac{1}{3} \mu$ .

Specimens examined:

North Carolina: Blowing Rock, G. F. Atkinson, 4200.

South Carolina: Santee River, Ravenel, Curtis Herb., 1716 (the type and cotype in Kew Herb. and Curtis Herb. respectively).

11. C. delitescens Burt, n. sp.

Plate 17. fig. 18.

Type: in Burt Herb.

Fructifications gregarious, cespitose, somewhat fleshy; pileus thin, convex, then umbilicate, dry, fibrillose, sepia-colored, the margin inrolled; stem equal, solid, glabrous, chamois-colored; hymenium even or sometimes obscurely lamelliform, chamois-colored; spores white, even, broadly ovoid,  $9 \times 7 \mu$ , borne four to a basidium.

Fructification 10-15 mm. high; pileus 5 mm. broad; stem 10-15 mm. long, 1 mm. thick.

Growing among mosses on very thin soil on rocks by waterfall. Vermont. August.

This species is intermediate between Cantharellus and Craterellus in its hymenial structure, but, as some of the specimens have the hymenium even and bearing mature spores, I include the species in Craterellus. The specimens are much smaller than those of C. calyculus and have the pileus becoming merely umbilicate. The little fructifications were well concealed among the mosses; I have found them but once.

Specimens examined:

n

Vermont: Falls of Lana, Lake Dunmore, E. A. Burt, type.

12. C. taxophilus Thom, Bot. Gaz. 37: 215-19. f. 1-8. 1904. Plate 17. fig. 21.

Illustrations: Thom, ibid. f. 1-8.

Type: in Cornell Univ. Herb., 15445.

Fructifications single, rarely gregarious, fleshy-membranaceous, entirely white when young, becoming pallid to ochraceous buff with age, drying cinnamon buff; pileus narrowly obconic, slightly viscid, the apex truncate, plane, or depressed and with a thin margin which is erect or expanded; stem solid, equal or tapering downward, flexuous, pruinose, with scattered white hairs at the base; hymenium even, becoming longitudinally rugose-wrinkled with age or upon drying; spores white, even, subglobose,  $3-4~\mu$  in diameter, borne four to a basidium.

Fructifications 1-3 cm. high; pileus 4-9 mm. broad; stem  $\frac{1}{2}$ -2 cm. long,  $\frac{1}{2}$ -1 mm. thick.

On rotten twigs and leaves under prostrate branches of *Taxus* canadensis. New York. October and November.

This delicate fungus was under observation by Dr. Thom

for a month and is described in detail and beautifully illustrated in connection with his original description in the work cited above. I reproduce merely some simple outline sketches of *C. taxophilus*; this is a very distinct species. The specimens were found in Fall Creek Gorge and nowhere except under prostrate branches of *Taxus*, yet they grew on rotting twigs and leaves of other species as well as on pieces of *Taxus*.

Specimens examined:

New York: Ithaca, C. Thom, Cornell Univ. Herb., 15445.

13. C. unicolor Rav. Grevillea 1: 148. 1873.

Plate 16. fig. 11, 12.

C. corrugis Peck, Bull. Torr. Bot. Club 26: 69. 1899.

Type: in Ravenel, Fung. Car. II. 26.

Fructifications solitary or cespitose, fleshy, with the flesh white, soft, soon shrinking and leaving the pileus hollow; pileus at first clavate, obtuse, flesh-colored tinted with violet, soon obconic or turbinate, broadly convex or truncate, and often abruptly cerebriform at the upper end, glabrous, ochraceous buff, drying Rood's brown to Natal-brown, the margin obtuse, corrugated by the hymenial wrinkles; stem short, equal or tapering downwards, colored like or a little paler than the pileus; hymenium wrinkled or corrugated, colored like the pileus; spores white,  $8-12 \times 4-6 \mu$ .

Fructifications 2-5 cm. high; pileus  $1\frac{1}{2}$ -5 cm. broad; stem  $1-2\frac{1}{2}$ 

cm. long, 5-8 mm. thick.

On ground in thin woods. Massachusetts, Pennsylvania,

and South Carolina. October to January.

This fungus presents strikingly the vagaries in the distribution of fungi. It was originally collected at Black Oak, South Carolina, in 1850, by Ravenel, in sufficient quantity so that he distributed the type collection in his exsiccati. Apparently, this fungus, whenever collected, was referred to other species until 1898, when members of the Boston Mycological Club found it in several localities in Massachusetts and it was adequately described by Peck, as C. corrugis, from specimens received from Dr. Francis. I have received no specimens of this species since that season; I searched for it in vain for several years in the adjoining state, Vermont. I have compared the specimens of C. corrugis, received from Dr. Francis, with Peck's

type and with the specimens of *C. unicolor* in five different copies of Ravenel's 'Fungi Caroliniani.' *C. corrugis* is certainly the same species as *C. unicolor*. It is very strange that in the interval of nearly half a century from the time of the original collection, *C. unicolor* did not attract attention from an intermediate station.

Specimens examined:

Exsiccati: Ravenel, Fung. Car. II. 26; Ell. & Ev., N. Am. Fungi, 1922a under the name C. pistillaris.

Massachusetts: Worcester, G. E. Francis, 61, 84, and collection dated Nov. 2, also the type (in Coll. N. Y. State) of C. corrugis; Lynn, H. Webster; Medford, Mrs. Page and Mrs. De Long, ex Herb. Boston Mycological Club, 420; Arlington Heights, E. A. Burt.

Pennsylvania: Trexlertown, W. Herbst, the C. clavatus of his 'Fungal Flora'; West Chester, B. M. Everhart, Ell. & Ev., N. Am. Fungi, 1922a.

South Carolina: Black Oak, Ravenel, 1406 (in Curtis Herb. and in Kew Herb.), and type, Ravenel, Fung, Car. II. 26.

14. C. pistillaris Fries, Epicr. 534. 1836-1838.

Plates 16, 17. figs. 13, 14.

Illustrations: Schæffer, Icon. Fung. pl. 169.—Harper, Mycologia 5: 263. pl. 95.

Fructifications gregarious, fleshy-spongy, drying sorghumbrown to fuscous; pileus somewhat clavate to turbinate or narrowly obconic, truncate, or somewhat convex, at first yellowish cinnamon, then becoming tinged with fuscous, the edge obtuse; stem solid, paler than the pileus, often bulbous at the base; hymenium corrugated and rugose-wrinkled, colored like the pileus, drying sorghum-brown to fuscous; spores even,  $10-12 \times 6-8 \mu$ .

Fructifications 6-12 cm. high; pileus  $2-3\frac{1}{2}$  cm. broad; stem 3-6 cm. long, 4-12 mm. thick.

On ground in woods under coniferous trees. New Hampshire, Vermont, and Michigan. August to October.

Specimens of this species have so nearly the coloration of C. unicolor that those, small and undeveloped, in a collection of C. pistillaris cannot readily be distinguished from partially developed specimens of C. unicolor; but with age, those of C.

unicolor—or at least some of them—have the pileus enlarge abruptly in diameter near the upper end and become abruptly globose-cerebriform on a slender stem, as shown in figs. 11 and 12, while *C. pistillaris* increases in length as well as in diameter, tapers downward more uniformly from the truncate upper end, and may have the stem bulbous at the base.

It is a vexed question with mycologists whether Craterellus pistillaris Fr. is Clavaria pistillaris L. The specimens which I refer to Craterellus pistillaris agree well with specimens of this species in Curtis Herbarium, collected at Upsala, Sweden, in 1853, and communicated by E. P. Fries. Pl. 16 fig. 13 is from a photograph, natural size, of these specimens. Their spores are 9 x 6 μ. The Friesian specimens have the same dark color as our American specimens. Only one of the former shows a bulbous tendency at the base of the stem; in this respect our specimens are more like the illustration of Schæffer, cited above. I believe, therefore, that we have Craterellus pistillaris Fr. in our flora. I have collected in mixed frondose woods in Missouri what I refer to Clavaria pistillaris as understood by European mycologists. As compared with the former species it is of softer structure, much paler in color, more regularly clavate in form, sometimes splitting at the apex. The illustrations of most European authors agree well in regard to Clavaria pistillaris. The colored figures of this species in Batsch, Bulliard, Sturm, Dufour, Flora Danica, Hussey, Krombholz, Quelet, and Sowerby present fructifications of the same habit and bright coloration which we have by Peck, Bull. N. Y. State Mus. 04: pl. 93. f. 1-4. and Mem. N. Y. State Mus. 4: pl. 66. f. 15-17.

Specimens examined:

Sweden: Upsala, E. P. Fries (in Curtis Herb.).

Austria: G. Bresadola.

New Hampshire: Shelburne, W. G. Farlow (in Mo. Bot. Gard. Herb., 4933).

Vermont: Middlebury, E. A. Burt.

15. C. palmatus Burt & Overholts, n. sp. Plate 17. fig. 19. Type: in Mo. Bot. Gard. Herb. and in Overholts Herb.

Fructifications gregarious or perhaps cespitose, fleshy-soft; pileus fawn-color shading into bone-brown towards the stem,

glabrous, flattened and ligulate at first, then spreading out laterally at the apex, and at length somewhat palmately cleft into 2-12 unequal, obtuse, finger-shaped branches; stem curved, solid, equal or somewhat tapering towards the base, bone-brown, sometimes swollen where attached to the substratum; hymenium even or but slightly venose, inferior, colored like the pileus; spores white, even, pyriform, tapering to the base, 6-8 x 3-4  $\mu$ .

Fructifications  $1-2\frac{1}{2}$  cm. high; pileus 3-15 mm. broad, 1 mm. thick; stem 8-15 mm. long,  $1-1\frac{1}{2}$  mm. thick.

On rotten chunks of wood in frondose woods. Ohio. June. All specimens of the collection except one have the pileus flabelliform; in this exceptional specimen, the pileus is narrowly turbinate, depressed, and with the finger-shaped branches arranged in a circle on the margin, pl. 17 fig. 19b. This species makes for Craterellus the same connection between the centralstemmed, cup-shaped type of pileus and the flabelliform type that Thelephora multipartita shows in Thelephora, and that is common in Stereum. The hymenium of the flabelliform specimens of Craterellus palmatus is so similar to the upper surface of the pileus in color and consistency that one cannot readily distinguish between these surfaces in the dried specimens. For these reasons, the present species cannot be referred to either Skepperia or Friesula, and it is of especial interest in showing that Craterellus has a natural section of species with flabelliform pileus. The spores of C. palmatus are noteworthy. Specimens examined:

Ohio: Oxford, L. O. Overholts, 1649, type (in Mo. Bot. Gard. Herb. and in Overholts Herb.).

16. C. dilatus Burt, n. sp. Plate 17. fig. 16.

Type: in Farlow Herb.

Fructifications single, fleshy; pileus flabelliform, somewhat triangular, glabrous, drying a dirty pinkish buff, the margin somewhat irregularly lobed, crisped, and curving upward; stem solid, equal, flexuous, drying Natal-brown, with white mycelium at the base; hymenium even, drying Isabella-color to clay-color; spores white, even, broadly ovoid, obtuse, 8–10 x 6–7  $\mu$ .

Dried fructification 4 cm. long; pileus 15 mm. long, 15 mm. broad,  $\frac{1}{2}$  mm. thick; stem  $2\frac{1}{2}$  cm. long, hardly 1 mm. thick.

On sandy ground in swamp. Florida. September.

Only a single fructification was collected; the description is based upon this dried specimen. The species is distinguished by its fan-shaped, triangular pileus and the comparatively long and slender stem. Its characters are those of a true *Craterellus* and yet such that we cannot regard it as a flabellate form of any other species.

Specimens examined:

Florida: Sorrento Swamp, R. Thaxter, type (in Farlow Herb.).

17. C. Humphreyi Burt, n. sp. Plate 17. fig. 22.

Type: in Burt Herb. and in Humphrey Herb.

Fructifications gregarious, fleshy, moderately tough and flexible, entirely white, usually with the pileus standing out horizontally at the apex of the erect stem; pileus reniform, dimidiate, sometimes clasping behind, convex, becoming plane or somewhat depressed, usually even, dry, minutely pubescent, the margin entire, even or slightly crisped; stem lateral, erect, often bent at right angles just before joining the pileus, cylindric below, equal, solid, pubescent; hymenium nearly even, sometimes radiately venose near the stem, brittle when fresh; spores white, even, subglobose,  $3\frac{1}{2}-4\frac{1}{2} \times 3\frac{1}{2} \mu$ .

Fructifications 3-7 cm. high; pileus 6 mm. - 2 cm. long,  $1-3\frac{1}{2}$  cm. broad,  $\frac{3}{4}$  mm. thick; stem  $2\frac{1}{2}$ -6 cm. long, 2 mm. thick.

On humus and among mosses in low swampy thicket. Washington. October.

The habit of this curious species is very suggestive of Hydnum auriscalpium; many of the specimens have the erect stem bent at right angles near the apex so that the pileus extends out in a horizontal plane. Sometimes the stem branches at its upper end and bears two pilei. The pubescence on the stem is rather coarse and is most abundant towards the base. All parts of the fructification were rather brittle in vegetative condition, and broke when bent too far. It is a connecting species between Craterellus and Arrhenia, but with the hymenium rather too even for Arrhenia, in my opinion.

Specimens examined:

Washington: Hoquiam, C. J. Humphrey, 1386, type.

Berkeley & Curtis, Jour. Linn. Soc. Bot. 10: 328, described three species of Craterellus from Cuba, which have been transferred to other genera by Patouillard, Bull. Soc. Myc. France 15: 193-94. pl. 9, as follows: C. spathularius to Skepperia and C. marasmioides and C. pulverulentus to Cymatella. I have received no collections referable to these genera and defer their consideration to the final part of my monograph in the hope that some specimens may be received in the meantime.

Craterellus canadensis Kl. ex Saccardo, Syll. Fung. 6: 519. 1888, was published by Berkeley, Ann. Nat. Hist. 3:380. 1839, under the name Cantharellus canadensis Kl. from a specimen in Hooker Herb. bearing manuscript notes by Klotzsch. The specimen was collected in Canada by Richardson. In connection with the original description, Berkeley noted that the nearest affinities of C. canadensis are with C. clavatus. In 1856, after studying the specimens in Herb. Schweinitz, Berkeley & Curtis, Jour. Acad. Nat. Sci., Phila. N. S. 3: 206. 1856, note that Cantharellus canadensis Kl. is apparently the same species as Cantharellus floccosus Schw. I have seen no specimens of C. canadensis and follow Berkeley's final disposition of the species.

(To be continued.)

# EXPLANATION OF PLATE PLATE 15

All figures of this plate have been reproduced natural size from photographs of dried herbarium specimens.

Fig. 1. Thelephora caspitulans. From authentic specimen in Curtis Herb., collected by Schweinitz in North Carolina.

Fig. 2. T. lutosa. From authentic specimen in Curtis Herb., collected by Schweinitz in North Carolina.

Fig. 3. T. dentosa. From cotype in Curtis Herb., collected in Cuba by C. Wright.

Fig. 4. T. perpleza. From type in Curtis Herb., collected in Cuba by C. Wright, 238. a shows a resupinate portion, and b, an ascending portion of the specimen.

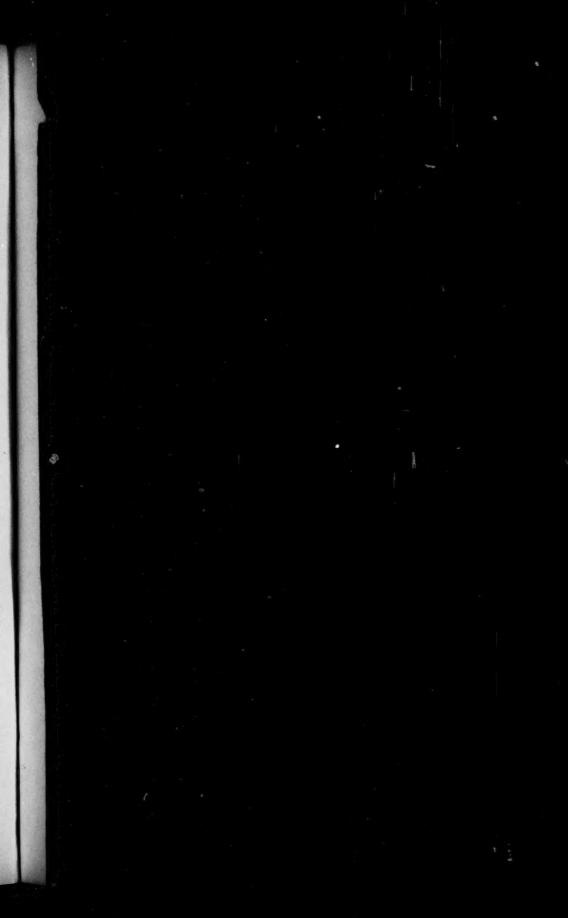
Fig. 5. T. cornucopioides. From specimen collected in Castleton Gardens, Jamaica, by F. S. Earle, 238.

Fig. 6. Craterellus clavatus. From specimen collected at Lake Dunmore, Vt.

Fig. 7. C. Cantharellus. From the cotype in Curtis Herb., 4539, of C. lateritius, collected in Alabama, by Peters.

Fig. 8. C. odoratus. From the cotype in Curtis Herb. of C. confluens, collected near Orizaba, Mexico, by Botteri, 6.

Fig. 9. C. odoratus. From the specimens in Curtis Herb., collected at Society Hill, S. Carolina, by Ravenel, 192.



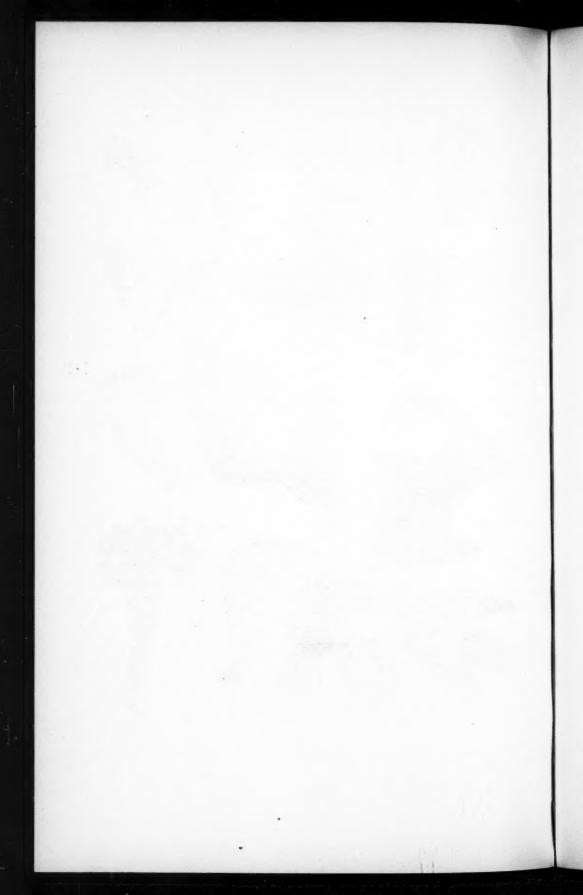


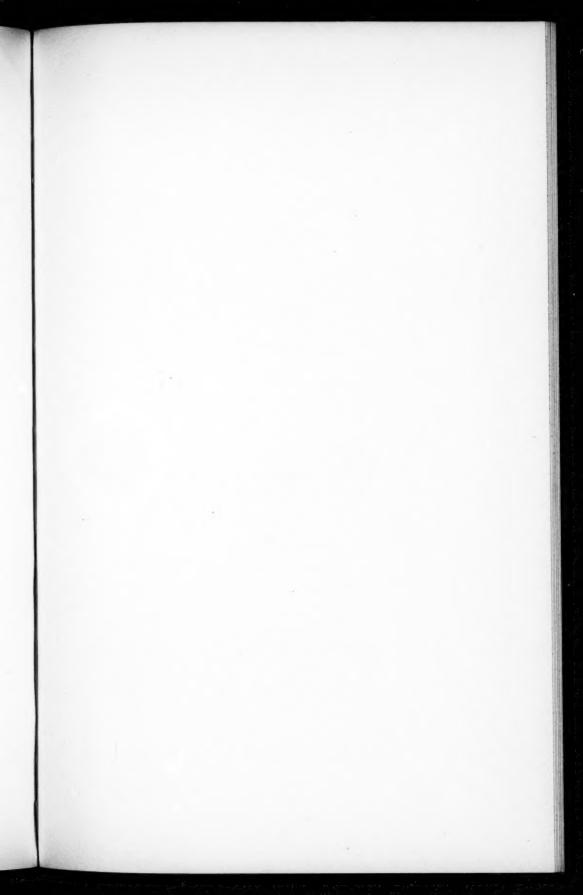
B



BURT-THELEPHORACEAE OF NORTH AMERICA

1. THELEPHORA CAESPITULANS.—2. T. LUTOSA.—3. T. DENTOSA.—4. T. PERPLEXA —5. T. CORNUCOPIOIDES. —6. CRATERELLUS CLAVATUS. —7. C. CANTHARELLUS. —8 and 9. C. ODORATUS.





## EXPLANATION OF PLATE PLATE 16

All figures of this plate have been reproduced natural size from photographs of dried herbarium specimens, but in the case of fig. 10 the specimens were moistened.

Fig. 10. C. odoratus. From specimens collected near St. Louis, Mo., by N. M. Glatfelter, 348. The rough dried specimens were moistened before being photographed. a shows a branched specimen; b, a fructification split longitudinally to show extent of depression of the pileus and the hollow stem; c, view of hymenium.

Fig. 11. C. unicolor. From authentic specimen in Curtis Herb., collected at Black Oak, S. Carolina, by Ravenel, 1406.

Fig. 12. C. unicolor. From specimen of C. corrugis collected at Medford, Mass., by Mrs. Page and Mrs. DeLong.

Fig. 13. C. pistillaris. From specimen in Curtis Herb., collected at Upsala, Sweden, by E. P. Fries.

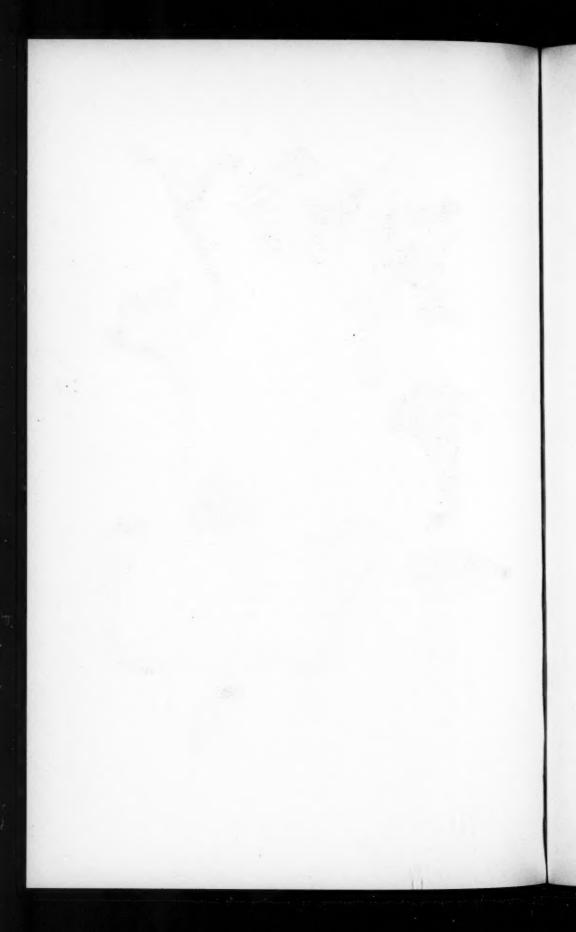


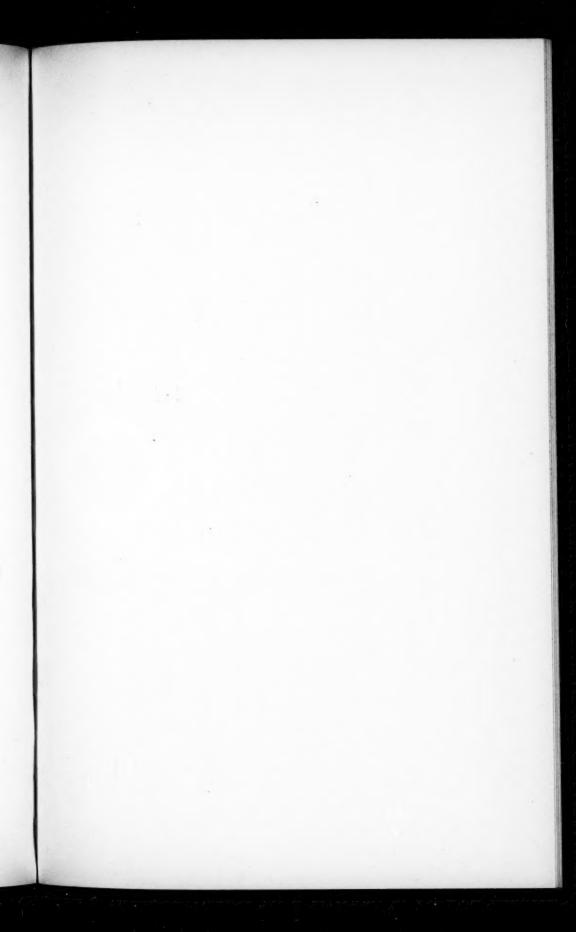




BURT—THELEPHORACEAE OF NORTH AMERICA

10. CRATERELLUS ODORATUS.—11 AND 12. C. UNICOLOR.—13. C. PISTILLARIS.





### EXPLANATION OF PLATE

#### PLATE 17

All figures are natural size. Figures 14-20-are from photographs of dried herbarium specimens, but which were moistened before being photographed in case of specimens used for figs. 15 and 17.

Fig. 14. C. pistillaris. From specimen collected under hemlock (Tsuga) tree, at Middlebury, Vt.

Fig. 15. C. ochrosporus. From type specimens in Mo. Bot. Gard. Herb., collected near St. Louis, Mo., by N. M. Glatfelter, 1253. a is split longitudinally to show the depth of depression of the pileus; b, side view.

Fig. 16. C. dilatus. From type in Farlow Herb., collected at Sorrento Swamp, Florida, by R. Thaxter. a shows upper surface of pileus, and b, the hymenium.

Fig. 17. C. cornucopioides. From specimen collected in Canada, by J. Macoun,

 Fig. 18. C. delitescens. From type specimens collected at Lake Dunmore, Vt.
 Fig. 19. C. palmatus. From type specimens in Mo. Bot. Gard. Herb. and Overholts Herb., collected at Oxford, Ohio, by L. O. Overholts, 1649. a shows specimens having flabelliform pileus, and b, a specimen with turbinate pileus.

Fig. 20. C. lutescens. a shows hymenium of specimen collected at Shelburne, New Hampshire, by W. G. Farlow, and b, upper surface of specimen collected at Lake Dunmore, Vt.

Fig. 21. C. taxophilus. From sketches of photographs of type specimens when in vegetative condition, collected at Ithaca, New York, by C. Thom.

Fig. 22. C. Humphreyi. From sketches of the type specimens when in vegetative condition, collected at Hoquiam, Wash., by C. J. Humphrey, 1386.

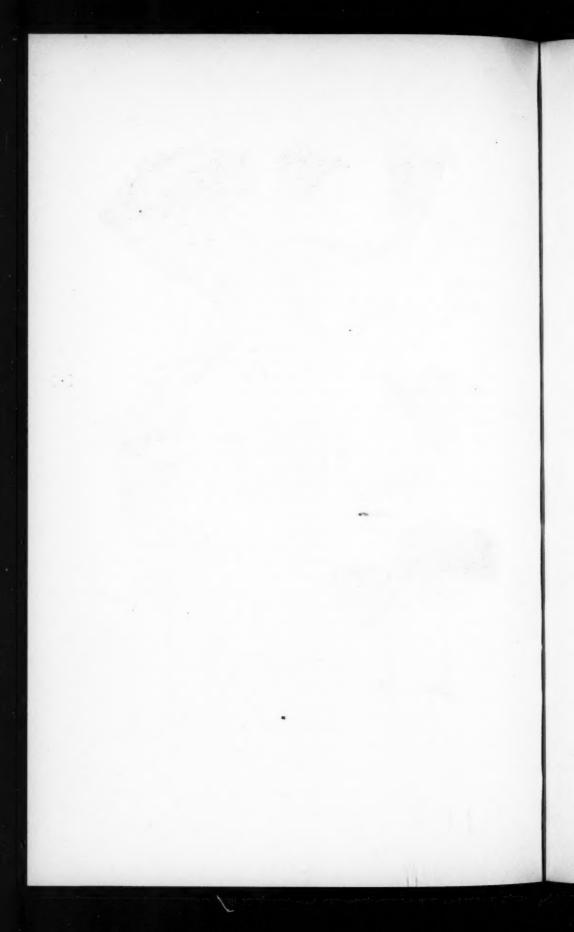






BURT-THELEPHORACEAE OF NORTH AMERICA

14. CRATERELLUS PISTILLARIS. — 16. C. OCHROSPORUS. — 16. C. DILATUS. — 17. C. CORNUCOPIOIDES. — 18. C. DELITESCENS. — 19. C. PALMATUS. — 20. C. LUTESCENS. — 21. C. TAXOPHILUS. — 22. C. HUMPHREYI.



# THE EFFECTS OF SURFACE FILMS ON THE RATE OF TRANSPIRATION: EXPERIMENTS WITH POTTED POTATOES

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In a previous report<sup>1</sup> we have presented data which is believed to justify the conclusion that an application of a surface film of Bordeaux mixture to the leaves of the castor bean or the tomato increases materially the rate of transpiration. The importance of a careful determination of various physiological effects of this spray mixture was suggested primarily by the increased vitality and yield exhibited by potatoes (Solanum tuberosum) treated with this fungicide during seasons when fungi and insects were unimportant factors. In our previous experiments the potato was not included, and it seemed most important, as a next step, to ascertain the effects of certain sprays upon the transpiration of this plant.

Experience has demonstrated that the potato may not be used satisfactorily in potometer experiments. Moreover, it was desired to arrange the experiment so that the transpiration quantities obtained might represent an interval of a week or more. On the other hand, it had been found as a result of our previous work with potted tomatoes that a very considerable amount of labor is required when it becomes necessary to add measured quantities of water every day to a series of fifty or more potted plants. Accordingly, for this and for other work proposed, a method was devised whereby we were able to employ a self-watering device based on a principle often used in the laboratory.

<sup>&</sup>lt;sup>1</sup> Duggar, B. M., and Cooley, J. S. The effect of surface films and dusts on the rate of transpiration. Ann. Mo. Bot. Gard. 1:1-22. pl. 1. 1914.

The apparatus is shown in pl. 18. The rack, or support, is made of a single sheet of galvanized iron 18 cm. wide and 55 cm. long, these dimensions being adequate for a stand 33 cm. high. Besides cutting a hole in the upper part for the insertion of the neck of the bottle, the operation of making a stand will be clear from the plate and involves merely a few slits with the shears, the balance being accomplished by bending. Two or four rivets may be used if additional strength is required. With regard to other features of the apparatus it is well to note that (1) the shoulder of the flower pot rests on the rim of a tin cup somewhat deeper than the pot, the latter containing the immediate supply of water; (2) there is an inverted bottle with a capacity of about 1500 cc. serving as a reservoir of water and aspirator; and (3) the bottle is connected with the cup by glass and rubber tubing.

In setting up an experiment the exposed area of the pot (above the shoulder) and the soil are covered with paraffin or parawax; the cup is filled with water to such height that when the pot is inserted the water will rise to the height of about 2 cm. on the side of the pot, thus insuring adequate absorption; while a notch in the side of the cup makes it possible to introduce the rubber tube connecting with the bottle, this tube being adjusted to reach just below the new level of water in the cup. With a tube of proper diameter, the water level in the cup is kept practically constant so long as the bottle contains water. This apparatus, complete, may be quickly and sufficiently accurately weighed on the Troemner scales. To prevent upsetting, after arranging in the experimental area, it is well to make the stand secure by providing a small hole in the base, through which a bamboo stick may be thrust into the soil. this stake, also, for further support, the bottle may be fastened by cord or rubber band.

The device above described has saved much time and has enabled us to obtain a soil moisture content practically uniform in all the pots used in the experiment. It possesses the disadvantage of tending to maintain a moisture content which for long-term cultures is too high for the best growth of the potato. A slight modification of the method would seem to be practicable in several aspects of transpiration work.

Three weeks before the experiment began the plants were repotted, new 5-inch pots of good quality being used, and at the time of the installation of the experiment the drainage holes in the pots were carefully corked, so that all transfer of water would be through the porous walls. The potato plants employed were grown in the greenhouse during the early spring, but on April 20, about two weeks before the test was made, they were placed outside, to insure hardiness. When used, the plants were from 25 to 45 cm. high, each plant with from about 15 to 30 leaves. Some plants were blossoming, and tubers were forming.

The experiment embraced 7 series, or lots, of 10 plants each, sprayed with mixtures as follows: (1) strong Bordeaux. (2) control, no spray, (3) weak Bordeaux, (4) lime wash, (5) lime sulfur. (6) strong Bordeaux and lampblack, and (7) lime wash and lampblack. The strong Bordeaux (designated hereafter Bordeaux) contained 12 grams CuSO, and 14.4 grams CaO per liter of water, being approximately the 5-6-50 formula of agricultural practise. It was made up in the usual way. weak Bordeaux was one-half the strength of the stronger mixture. The lime wash was a Ca(OH)2 suspension consisting of 60 grams of CaO per liter of water. A commercial preparation of lime sulfur was used, and this was diluted, as usual, to The Bordeaux-lampblack and the lime-washlampblack preparations were made by rubbing into small quantities of the Bordeaux and lime wash 5 and 10 grams respectively of lampblack, then diluting to one liter.

The method of selecting the plants for the different lots was precisely that described in the previous report, that is, selecting at one time 7 plants (as many as there were lots) between which there could be little or no choice, and distributing these at random, 1 to each lot until each included 10 plants. All plants (except controls) were sprayed on May 5, but a rain that night, before protection was provided, necessitated respraying the following day. After spraying, the plants were placed on the stands and each connected with its water supply. They were arranged on an exposed lawn, each lot occupying a row, with the plants 4 feet apart. Moreover, several rows of potted potatoes were arranged around the entire area in order that all

plants in the experimental area might have equal exposure. Over the experimental plot a frame was provided, so that the whole area might be protected by tarpaulins in case of rain. Fortunately, however, no rain occurred during the period of the experiment.

After a preliminary exposure of 24 hours, which enabled us to determine that the 70 plants of the experimental area were in good condition, the initial weighings were made. A definite order was established, this being crosswise of the different lots. The same order was observed at the close of the period, and similarly in the second period a consistent scheme was followed, in order that the time interval might be as uniform as possible. After the weighings at the close of the first period, all plants were discarded which showed any signs of weakness or injury arising from the conditions of the experiment. It should be stated, too, that these conditions were taxing. The weather was bright and warm, the pots were severely exposed, and, as already noted, the water content of the pots was necessarily fairly high. With the plants remaining in a condition apparently normal and vigorous from the first period, a second "run" was made, the latter including from 4 to 7 plants in

TABLE SHOWING WATER LOSS AND GREEN WEIGHT OF THE PLANTS

Lot	Film covering	1st period, May 6–11, 10 plants			2nd period, May 11–15, 7 plants		
		Ave. water loss per plant	Ave. green weight per plant	Water loss per g., green weight	Ave. water loss per plant	Ave. green weight per plant	Water loss per g., green weight
1	Bordeaux, strong	526.6	50.3	10.46	463.3	55.0	8.42
2	Control	413.8	61.0	6.78	433.3	63.1	6.86
3	Bordeaux, weak	642.4	60.9	10.54	574.0	61.7	9.30
4	Lime wash	584.5	70.7	8.27	613.6	76.3	8.04
5	Lime sulfur	443.0	62.8	7.06	450.7	70.0	6.44
6	Bordeaux and lamp- black	792.1	66.1	11.97	653.0	75.2	8.69
7	Lime and lampblack	596.6	58.3	10.20	585.6	66.8	8.78

each lot. In selecting plants for this second period, the size factor was again taken into consideration, as far as possible.

More stress should, however, be laid upon the data from the first period. The green weights of the plants discarded at the close of the first period were taken immediately, while those plants used in the second period could not be weighed until the close of that interval. This small interval of time, however, could cause no material change in the weights. In the accompanying table there are given in grams the average water loss per plant, the average green weight per plant, and the water loss per gram of green matter.

From the data exhibited it is obvious that with potted potatoes, as with castor bean leaves and potted tomatoes in our earlier experiments, there is a marked acceleration of transpiration induced by spraying with Bordeaux mixture, as also with some other films. Of the several films employed, lime sulfur alone yields an average water loss comparable with that of unsprayed plants. Of all lots showing increased transpiration those treated with weak Bordeaux and lime wash were in some respects most satisfactory, inasmuch as the plants used, like those in the control, were, in general, in very good condition throughout the period of the experiment. On the other hand, those treated with the stronger Bordeaux, the Bordeaux and lampblack, and the lime and lampblack gave, towards the close of the periods, evidences of the injurious effects of the increased transpiration (apparently) upon the vitality of the plants. These statements may not seem to be in entire accord with the figures presented, for during the second period of the experiment, for example, the transpiration quantity is relatively greatest in the case of those plants sprayed with weak Bordeaux mixture. Nevertheless, our observations enabled us to predict that certain lots, especially numbers 1 and 6, would give in the second period, particularly, transpiration values less than might be anticipated. The smaller quantities in the lots referred to, as contrasted with the weak Bordeaux, are to be explained, in fact, as a direct result of incipient wilting and slight injury, brought about by the higher transpiration capacity induced under conditions already accentuating transpiration.

It is believed, in the first place, that the experiments here reported confirm our earlier conclusion, namely, that a film of Bordeaux mixture facilitates water loss; but, in the second place,

treatment with a fairly thick lime wash or lime wash and lampblack also increases the transpiration rates, the latter more than the former. Lampblack added to Bordeaux seems also to give a higher rate than the Bordeaux alone. It is to be emphasized. however, that the strength of the lime wash employed is four times as great as the lime in the stronger Bordeaux mixture: likewise, more lampblack is used with the lime wash than with the Bordeaux. It seems to be definitely established that certain specific characters of the film are important, but these results suggest, further, that the additional quality of color is a factor requiring consideration. The fact that injury may result from the accelerated transpiration induced by a heavy film of Bordeaux under the conditions of our experiment does not mean that under normal conditions of growth in the field a benefit may not accrue to certain plants-from factors associated with a high transpiration rate.

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## EXPLANATION OF PLATE PLATE 18

View of the apparatus (with tomato plant) by means of which watering was automatically controlled. It has been found convenient to have both stand and cup painted green. For description see text, p. 322.







DUGGAR AND COOLEY - TRANSPIRATION



